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(54) Title: **NOVEL GLUCANS AND NOVEL GLUCANSUCRASES DERIVED FROM LACTIC ACID BACTERIA**

(57) Abstract: The invention pertains to novel glucans capable of being produced by glucosyltransferase activity of a lactic acid bacterium on a sucrose substrate, the glucan having an average molecular weight between 10 kDa and 1 GDa, consisting essentially of  $\alpha(1,3)$ - and  $\alpha(1,6)$ -linked anhydroglucose units (AGU) and to glucansucrases capable of producing these glucans from sucrose. The glucans have thickening and anti-corrosive properties. The glucans can be chemically modified.

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## Novel glucans and novel glucansucrases derived from lactic acid bacteria

[0001] The present invention is in the field of enzymatic production of biomolecules. The invention is particularly concerned with novel glucans derived from lactic acid bacteria, with novel glucosyl-transferases derived from such bacteria and with a process for  
5 production of new and useful glucans from sucrose.

### Background of the invention

[0002] Several bacteria are known to produce exopolysaccharides, i.e. polysaccharides secreted into the culture medium. Well-known examples of bacterial exopolysaccharides include xanthan from *Xanthomonas campestris*, gellan from *Sphingomonas paucimobilis*  
10 and pullulan from *Aureobasidium pullulans*. Lactic acid bacteria known to produce exopolysaccharides include *Leuconostoc mesenteroides* strains producing dextrans,  $\alpha(1\rightarrow 6)$ -linked poly-anhydroglucose, and alternans i.e. poly-anhydroglucoses having alternating  $\alpha(1\rightarrow 6)$  and  $\alpha(1\rightarrow 3)$ -linkages, oral *Streptococcus* strains producing glucans responsible for dental plaque formation, and a particular *Lactobacillus reuteri* strain producing  
15  $\alpha(1,6)$ - and  $\alpha(1,4)$ -linked anhydroglucose (Van Geel-Schutten, *et al.*, *Appl. Environ. Microbiol.* (1999) 65, 3008-3014). The properties of exopolysaccharides depend on the type of monosaccharide units, the type of linkages, the degree and type of branching, the length of the polysaccharide chain, the molecular weight and the conformation of the polymers.

[0003] Argüello-Morales *et al.* (*FEMS Microbiol. Lett.* 182 (2000) 81-85) describe an alternansucrase from *Leuconostoc mesenteroides* NRRL B-1355. Monchois *et al.* (*Gene* 182 (1996) 23-32; *FEMS Microbiol. Lett.* 159 (1998) 307-315) for instance describe two different dextransucrases from *Lc. mesenteroides* NRRL B-1299. A method for selecting *Leuconostoc mesenteroides* strains that produce a high proportion of alternan to dextran is  
25 described in US 5,789,209. The prior art does not disclose or suggest other lactic acid bacteria than *Leuconostoc* or *Streptococcus* that are capable of producing glucans having both  $\alpha(1\rightarrow 6)$  and  $\alpha(1\rightarrow 3)$ -linkages.

### Summary of the invention

[0004] Several lactic acid bacteria strains were found, according to the invention, to be  
30 capable of producing a particular class of glucans. These glucans have in common that their anhydroglucose units (AGU) are linked  $\alpha(1,3)$ - and/or  $\alpha(1,6)$ -glucosidic bonds, i.e. they are  $\alpha$ -glucans largely or completely devoid of  $\alpha(1,4)$ -bonds. These glucans may be of

the alternan (alternating  $\alpha(1,3)$  and  $\alpha(1,6)$  linkages), mutan (mixed  $\alpha(1,3)$  and  $\alpha(1,6)$  linkages, usually  $\alpha(1,3)$  predominant) or dextran (mainly  $\alpha(1,6)$  linkages, some  $\alpha(1,3)$ ) type, or other type. The glucans can be produced from sucrose, using sucrase enzymes which are active in the lactic acid bacteria. They can be produced on a large scale and isolated in a commercially feasible way, as the glucans are produced outside the bacterial cell, or even in the absence of the bacteria, using isolated sucrase enzymes. The glucans are produced by food-grade strains and have interesting properties, such as prebiotic utility or thickening of water-based compositions.

[0005] The invention is concerned with these novel glucans, with the lactic acid bacterial, especially *Lactobacillus* strains and their enzymic proteins that produce these glucans from sucrose, as well as with methods for producing the glucans using the strains and/or their enzymes, with nucleotide sequences encoding these enzymic proteins which convert sucrose, with the use of the glucans as thickeners, prebiotics, anticorrosives, etc., and as starting materials for modified glucans.

#### 15 *Description of the invention*

[0006] The invention pertains to *Lactobacillus* strains containing a glucosyltransferase (glucansucrase) capable of producing a glucan having at least 10 anhydroglucose units (AGU) having a backbone consisting essentially of  $\alpha(1,3)$ - and/or  $\alpha(1,6)$ -linked AGU, in the presence of sucrose. Such strains can be found among current sources of *Lactobacilli*, such as food sources, silage, mammalian samples etc. These strains containing the glucosyltransferases and producing the glucans can be identified by isolating *Lactobacillus* strains from these sources, growing them on sucrose and analysing the polysaccharide product using suitable analytical methods such as chromatography. The genes encoding these glucosyltransferases can be identified by amplifying nucleotide sequence fragments of the strain using primers based on known glucosyltransferase genes and retaining the positive strains (see examples). Several glucan-producing strains were isolated and identified from different sources and different *Lactobacillus* species, such as *Lb. reuteri*, *Lb. fermentum*, *Lb. sake* and *Lb. parabuechneri* or related species. The glucosyltransferases from these glucan-producing strains were also identified and, completely or partly, sequenced (see Examples).

[0007] The novel glucans of the invention are capable of being produced by glucosyltransferase (glucansucrase) activity of a lactic acid bacterium on a sucrose donor substrate. The glucans have an average molecular weight between 10 kDa and 1 GDa, and

consist essentially of  $\alpha(1,3)$ - and/or  $\alpha(1,6)$ -linked anhydroglucose units (AGU), to which side-chains also consisting of  $\alpha(1,3)$ - and/or  $\alpha(1,6)$ -linked AGU may be attached.

[0008] In particular, the glucans according to the invention either comprise 15-80% of  $\alpha(1,3)$ -linked AGU, 2-80%, especially 4-80% and more especially 15-80% of  $\alpha(1,6)$ -linked and 2-25% of  $\alpha(1,3,6)$ -linked (branching) AGU, or 80-99% of  $\alpha(1,6)$ -linked AGU and 1-20% of  $\alpha(1,3)$ -linked or  $\alpha(1,3,6)$ -linked (branching) AGU, in particular 1-15% of  $\alpha(1,3)$ -linked AGU and 5-15% of  $\alpha(1,3)$ - and  $\alpha(1,3,6)$ -linked units taken together. Thus, the invention covers a glucan having an average molecular weight of 50 kDa to 1 MDa and comprising 25-50%, especially 29-39% of  $\alpha(1,3)$ -linked AGU, 20-45%, especially 30-40% of  $\alpha(1,6)$ -linked AGU, 5-25%, especially 3-13% of  $\alpha(1,3,6)$ -linked AGU and 6-30% of terminal AGU. Furthermore, the invention pertains to a glucan having an average molecular weight of 10-50 MDa and comprising 15-26%  $\alpha(1,3)$ -linked AGU, 30-50% of  $\alpha(1,6)$ -linked AGU, 5-20% of  $\alpha(1,3,6)$ -linked AGU and 5-35% of terminal AGU. Also, in another embodiment the invention covers a glucan having an average molecular weight of 1-50 MDa and comprising 40-60% of  $\alpha(1,3)$ -linked AGU, 2-20%, especially 2-12% of  $\alpha(1,6)$ -linked AGU, 10-25% of  $\alpha(1,3,6)$ -linked AGU and 10-30% of terminal AGU. In yet another embodiment, the invention comprises a glucan having an average molecular weight of 10-50 MDa and comprising 80-99%, especially 88-99% and more especially 90-99% of  $\alpha(1,6)$ -linked AGU, or 80-90% of  $\alpha(1,6)$ - and 1-10% of  $\alpha(1,3)$ -linked AGU, the remainder being 1,3,6 linked and terminal AGU.

[0009] The invention also concerns the enzymes originating from lactic acid bacteria, or from recombinant sources, capable of producing the glucans described above starting from sucrose. The enzymes are new and they can be classified as glucansucrases or glucosyltransferases. Their partial sequence information is given below in SEQ ID No's 1-10. More complete sequence information is given in SEQ ID No's 11-22. Proteins according to the invention comprise an amino acid sequence exhibiting at least 70%, preferably at least 80%, most preferably at least 90%, amino acid identity with any one of the amino acid sequences of SEQ ID No. 2, 4, 8, 10, 12, 14, 16, 18, 20 and 22 or of stretches of at least 221-224 amino acids thereof, or at least 100 contiguous amino acids exhibiting at least 80%, preferably at least 90%, amino acid identity with these sequences. Further preferred sequences are indicated in the description of the alignment figure given below.

[0010] The enzymes can be used as such for producing the glucans described above, or for producing oligosaccharides and polysaccharides having a similar  $\alpha(1,3)$  and/or  $\alpha(1,6)$  linked glucan structure. Their genes can also be incorporated in suitable host organisms, to produce alternative glucan-production systems. The invention also pertains to such  
5 recombinant, preferably food-grade microorganisms, e.g. bacteria, especially lactic acid bacteria, yeasts, fungi etc., containing the genes of the glucansucrases described above and being capable of expressing the glucansucrases.

[0011] The invention also pertains to a process of producing a glucan as described above. This glucan can be produced by a *Lactobacillus* strain as described above, or by a  
10 recombinant micro-organism expressing the glucosyltransferase according to the invention or by an isolated glucosyltransferase according to the invention and a suitable glucose source such as for instance sucrose. The glucosyltransferase may be isolated by conventional means from the culture of a glucosyltransferase-positive lactic acid bacterium, especially a *Lactobacillus* species, or from a recombinant organism expressing  
15 the glucosyltransferase gene.

[0012] The glucan and the gluco-oligosaccharides produced by the *Lactobacillus* strains can be recovered from the culture supernatant of *Lactobacillus* strains described above, containing the glucosyltransferase according to the invention. The glucan can comprise at least 20, up to about 100,000  $\alpha$ -anhydroglucose units with the unique structure described  
20 above.

[0013] The glucan-producing enzymes according to invention, or at least the most preferred ones, are constitutive in the *Lactobacillus* strains, in that they are always present. This is contrast to most glucan (dextran-) producing *Leuconostoc* strains of the prior art, wherein the enzymes are only expressed upon growth in the presence of sucrose.  
25 This allows a more efficient production of glucans by the microorganisms of the invention.

[0014] The glucans according to invention have a variety of useful properties. They are suitable as prebiotics, and thus they can be incorporated in nutritional or pharmaceutical compositions intended for improving the condition of the gastrointestinal tract. For this  
30 purpose, they can be used as such or in the form of their oligosaccharides. They can also be combined with other poly- or oligosaccharides, such as fructans, galactans, xylans, arabinans, mannans, indigestible glucans and hetero-oligosaccharides, or with probiotic micro-organisms, including the lactic acid bacteria from which the glucans originate, resulting in synbiotic compositions. The glucans and their shortened homologues are also

useful as bioactive agents, e.g. as immunomodulators, anti-ulcer agents and cholesterol-lowering agents.

[0015] The glucans are also useful as thickening agents. As such they can be incorporated in foodstuffs such as beverages, sauces, dressings, dairy products, in amounts of from 1 g/l to about 100 g/l, especially about 10 to 50 g/l.

[0016] The glucans of the invention are furthermore useful as anticorrosion agents, for example for the protection of ship hulls. For that purpose, they may be applied in the form of solutions or suspensions, by spraying, coating, dipping and other techniques known in the art of corrosion control.

[0017] The glucans can be used as such. They can also be modified by physical or chemical means. Suitable examples of chemical modification include oxidation, especially 2,3- or 3,4-oxidation using periodate or hypohalite, in glucans having  $\alpha$ -1,6 linkages, or 6-oxidation using nitroxyls with peracid or hypohalite in glucans having  $\alpha$ -1,3 linkages. Hypohalite oxidation resulting in ring-opened 2,3- or 3,4-dicarboxy-anhydroglucose units (see e.g. EP-A-427349), while periodate oxidation results in ring-opened 2,3- or 3,4-dialdehyde-anhydroglucose units (see e.g. WO 95/12619), which can be further oxidised to (partially) carboxylated units (see e.g. WO 00/26257). Nitroxyl-mediated oxidation using hypochlorite or a peracid results in 6-aldehyde- and 6-carboxy-anhydroglucose units (see e.g. WO 95/07303).

[0018] The oxidised glucans have improved water-solubility, altered viscosity and a retarded fermentability and can be used as metal-complexing agents, detergent additives, strengthening additives, bioactive carbohydrates, emulsifiers and water binding agents. They can also be used as starting materials for further derivatisation such as cross-linking and the introduction of hydrophobes. Oxidised glucans coupled to proteins can be used as emulsifiers and stabilisers. The oxidised glucans of the invention preferably contain 0.05-1.0 carboxyl groups, more preferably 0.2-0.8 carboxyl groups per anhydroglucose unit, e.g. as 6-carboxyl groups on 1,3-linked units.

[0019] When modified glucans with high proportion of carboxyl groups are desired, two oxidation processes can be combined or an oxidation can be combined with e.g. carboxymethylation (see below). Thus, an  $\alpha$ -(1,3/1,6)-glucan having a degree of substitution (DS) for carboxyl groups between 0,3 and 1,0 can be conveniently prepared by first nitroxyl-mediated oxidation, resulting in 1,3-substituted units being oxidation to glucuronic acid units, followed by e.g. periodate and chlorite oxidation, resulting in 1,6-substituted units\* being converted to ring-opened dicarboxy-substituted units. The order

of processes can also be inverted, or one oxidation process, such as nitroxyl-mediated 6-oxidation can be combined with carboxymethylation. Also, by appropriate adaptation of the oxidation processes mixed aldehyde-containing and carboxyl-containing polymers can be obtained.

- 5 [0020] Other useful modifications are alkylation, acylation, hydroxyalkylation, amino-alkylation, carboxyalkylation, phosphorylation, sulphatation, as well as physical and chemical crosslinking. Phosphorylation (see: O.B. Wurzburg (1986), Modified Starches: properties and uses. CRC Press Inc., Boca Raton, 97-112) can be achieved by dry heating glucans with a mixture of monosodium and disodium hydrogen phosphate or with tripoly-  
10 phosphate. The phosphorylated glucans are suitable as wet-end additives in papermaking, as binders in paper coating compositions, as warp sizing-agents, and as core binders for sand molds for metal casting. Acylation, especially acetylation or propionylation using acetic or propionic anhydride respectively, results in products suitable as bleaching assistants and for the use in foils. Acylation with e.g. alkenyl succinic anhydrides or  
15 (activated) fatty acids results in surface-active products suitable as e.g. surfactants, emulsifiers, and stabilisers. Crosslinking, e.g. by coupling oxidised derivatives, or by reaction with a crosslinking agent such as triphosphoric acid, epichlorohydrine or a dialdehyde, can be used to adjust the physical properties of the glucans, e.g. to enhance their water-binding or thickening capacities.
- 20 [0021] Hydroxyalkylation is commonly performed by base-catalysed reaction with alkylene oxides, such as ethylene oxide, propylene oxide or epichlorohydrin; the hydroxy-alkylated products have improved solubility and viscosity characteristics. Carboxy-methylation is achieved by reaction of the glucans with monochloroacetic acid or its alkali metal salts and results in anionic polymers suitable for various purposes including  
25 crystallisation inhibitors, and metal complexants. Amino-alkylation can be achieved by reaction of the glucans with alkylene-imines, halo-alkyl amines or amino-alkylene oxides, or by reaction of epichlorohydrine adducts of the glucans with suitable amines. These products can be used as cationic polymers in a variety of applications, especially as a wet-end additive in paper making to increase strength, for filler and fines retention, and to  
30 improve the drainage rate of paper pulp. Other potential applications include textile sizing and wastewater purification. The above mentioned modifications can be used either separately or in combination depending on the desired product. Furthermore, the degree of chemical modification is variable and depends on the intended use. If necessary 100% modification, i.e. modification of all anhydroglucose units can be performed. However,

partial modification, e.g. from less than 1 (e.g. 0.2) modified anhydroglucose unit per 100 units up to higher levels, will often be sufficient in order to obtain the desired effect.

[0022] Another suitable type of derivatives is formed by hydrolysates of the present glucans. Hydrolysis can be performed in a controlled manner in a way known per se, using e.g. dilute acid or glucanolytic enzymes, especially  $\alpha$ -1,3-glucanases or  $\alpha$ -1,6 glucanases. Hydrolysis results in polysaccharides of reduced chain length (degree of polymerisation, DP, of more than 20) or oligosaccharides (DP of less than 20).

[0023] The invention also relates to gluco-oligosaccharides containing the characteristic structure of the glucan described above. These can be produced using an isolated glucansucrase according to the invention or a *Lactobacillus* strain, or a recombinant micro-organism containing (a part of) a glucosyltransferase according to the invention. Gluco-oligosaccharides thus produced can be used as prebiotics and probiotics. The production of the gluco-oligosaccharides is different from the glucan synthesis reaction. In addition to sucrose, the substrate of the glucansucrase, an acceptor molecule such as maltose or lactose can be used as an acceptor, to synthesise oligosaccharides. Consecutive attachment of glucose units in a manner determined by the particular glucansucrase results in  $\alpha$ (1,3)- and/or  $\alpha$ (1,6)-linked gluco-oligosaccharides, the chain length of which can be determined by selecting the appropriate reaction conditions. Longer reaction times, higher sucrose levels and lower acceptor levels will usually result in relatively long chains, e.g. having a degree of polymerisation (DP) of more than 10, up to several hundreds if desired, while shorter reaction times, lower sucrose levels and higher acceptor levels will result in relatively short chains, e.g. with a DP from about 3 up to 10 or higher. Another way of producing gluco-oligosaccharides is by hydrolysis of the glucan described above. This hydrolysis can be performed by known hydrolysis methods such as enzymatic hydrolysis with enzymes such as amylase, dextranase or pullulanase or by acid hydrolysis. The produced gluco-oligosaccharides contain at least one 1,6- or one 1,3-glucosidic link to be used as prebiotics.

[0024] The invention also relates to a probiotic or synbiotic composition containing a *Lactobacillus* strain capable of producing a glucan and/or gluco-oligosaccharide according to the invention. The strain may also produce another poorly digestible poly- or oligosaccharide, such as a fructan. The probiotic or synbiotic compositions of the invention may be directly ingested with or without a suitable vehicle or used as an additive in conjunction with foods. They can be incorporated into a variety of foods and beverages including, but not limited to, yoghurts, ice creams, cheeses, baked products



such as bread, biscuits and cakes, dairy and dairy substitute foods, confectionery products, edible oil compositions, spreads, breakfast cereals, juices and the like.

[0025] Furthermore, the invention pertains to a process of improving the microbial status in the mammalian colon comprising administering an effective amount of a *Lactobacillus* strain capable of producing a glucan and/or gluco-oligosaccharide according to the invention. Furthermore, a process of improving the microbial status of the mammalian colon comprising administering an effective amount of a glucan or gluco-oligosaccharide according to the invention is also a part of the present invention.

## 10 **Examples**

### **General**

The various lactic acid bacterial strains were isolated from a variety of sources, including fermented foods, the gastrointestinal tract of various human or animal species, and silage.

#### 15 **Example 1: Identification and nucleotide sequence of glucansucrase/glucosyltransferase genes from lactobacilli**

The glucansucrase genes were identified by amplification with PCR using degenerated primers (GTFrev, 5' ADRTC NCCRT ARTAN AVNYK NG 3' and GTFforw, 5'-GAYAA YWSNA AYCCNRYNGT NC-3'; N = A, C, G or T, Y = T or C, K = G or T, W = A or T, S = C or G, R = A or G), based on conserved amino acid sequences of different published glucansucrase genes. An amplification product with the predicted size of about 660 bp was obtained and cloned in *Escherichia coli* Top 10 using pCR-XL-TOPO (Invitrogen). Sequence analysis confirmed that part of a *gtf* gene had been isolated. The 660 bp amplified was used to design primers for inversed PCR. For inverse PCR chromosomal DNA was digested with 10 different enzymes ligated, yielding circular DNA molecules. PCR with the diverging primers with the circular ligation products as template yielded amplicons of various sizes, those products were cloned into pCR-XL-TOPO (Invitrogen) and sequenced (GATC, Konstanz, Germany). If necessary additional inverse PCR reactions were carried out to obtain the complete gene(s). Both strands of the entire glucansucrase genes were sequenced twice.

#### 30 **Example 2: Isolation and identification of $\alpha$ -(1,6) glucan and a glucansucrase from *Lactobacillus reuteri* strain 180**

*L. reuteri* strain 180 was deposited as LMG P-18389 at the BCCM/LMG Culture Collection at Gent, Belgium. The strain was grown in 18 litres of MRS-s medium (in g per kg): yeast extract (22), sodium acetate trihydrate (5), sodium citrate dihydrate (2.42), ammonium chloride (1.32), dipotassium hydrogen phosphate (2), magnesium sulphate heptahydrate (0.2), manganese sulphate heptahydrate (0.05), sorbitan mono-oleate (1), vitamins (in mg per kg: B1: 14.4, B2: 3.6, B3: 72, H 0.216), sucrose (100), tap water

(remainder), for 21 h at 37°C under anaerobic conditions (pH 5.5). See also: Van Geel-Schutten et al., Appl. Microbiol. Biotechnol. (1998) 50, 697-703. During growth, 13 g/l polysaccharide was produced. This polysaccharide was isolated as described in the reference cited above. The monosaccharide composition of the polysaccharide was determined by hydrolysis of the soluble part of the polysaccharide and high-performance anion-exchange chromatography. It was characterised as a glucan. This glucan was not formed when the strain was grown on glucose instead of sucrose. Methylation analysis (Van Geel-Schutten et al. 1999) revealed the presence of 17-24%  $\alpha(1,3)$ -linked glucosyl units, 34-44% of  $\alpha(1,6)$ -linked glucosyl units, 7-15% of  $\alpha(1,3,6)$ -linked glucosyl units and 7-35% of terminal glucosyl units. The average molecular weight of the glucan was determined to be  $3.6 \times 10^7$  Da and the Rg was 45 nm.

The average molecular weight of the polysaccharide was established using the SEC-MALLS system: 0.0522 g of the glucan was dissolved in 10 ml DMSO/water (90/10) and heated for 1 hour at 80°C, filtered through a 0.45  $\mu$ m filter and injected on the SEC-MALLS system and analysed using the following conditions:

Eluent:	DMSO/water (90/10) with 0.1 M NaNO <sub>3</sub>
Flow rate:	0.5 ml/min
Injection volume:	0.247 ml
Column:	PLgel Guard, mixed-A and mixed-D
Temperature:	90°C

Detection: MALLS (DAWN-DSP), 50°C, A<sub>2</sub>=0, dn/dc=0.074, F2 cell, RI; SDS PAGE followed by PAS-staining (Van Geel-Schutten et al. 1999) revealed the presence of an extracellular sucrase with a molecular weight of about 190 kDa. Part of the gene encoding the sucrase enzyme was isolated using PCR techniques and sequenced. On the deduced amino acid sequence of the fragment, high homologies were found with other glucan-sucrases. This partial sequence information is given in SEQ ID No. 1 (DNA) and 2 (protein). Full sequence information is given in SEQ ID No's. 11 and 12.

The glucan produced by *L. reuteri* strain 180 has been tested for application on ship hulls for the prevention of corrosion (see Example 8).

**Example 3: Isolation and identification of  $\alpha(1,6/1,3)$  glucan and a glucansucrase from *Lactobacillus reuteri* strain ML1**

*L. reuteri* strain ML1, deposited as LMG P-20347 at the BCCM/LMG Culture Collection at Gent, Belgium, was grown overnight under anaerobic conditions at 37°C on MRS supplemented with sucrose (see Example 2). The cells were removed by centrifugation and two volumes of ethanol were added to the supernatant. The precipitated polysaccharides were harvested by centrifugation and resuspended in 2-3 liters of demi water and precipitated again with two volumes of ethanol. The glucan produced by this strain (7 g) was characterised by methylation analysis and monosaccharide composition analysis as

described in Example 2. The polymer was found to consist of 48-53% of  $\alpha(1-3)$  linked glucosyl units, 3-8% of  $\alpha(1-6)$  linked glucosyl units, 12-20% of  $\alpha(1-3-6)$  linked glucosyl units (branching units) and 20-30% of 1-linked (terminal) glucose units. The glucans were not produced during growth on glucose. The average molecular weight of the polysaccharide was established to be  $7.6 \times 10^6$  Da using the SEC-MALLS system as described in example 2. These were the first examples of the production of mutan-like polymers by lactobacilli. The glucan produced by *L. reuteri* strain ML1 has been tested for application as anticorrosive agent and showed excellent utility for the prevention of corrosion e.g. on ship hulls.

SDS PAGE followed by PAS-staining (Van Geel-Schutten et al. 1999) revealed the presence of an extracellular sucrase with a molecular weight of about 190 kDa. It was found that this strain produces two glucansucrases. Sequence information for these sucrase is given in SEQ ID No's 13 and 14 (ML1) and 15 and 16 (ML4).

**Example 4: Isolation and identification of  $\alpha(1,6/1,3)$  glucan and a glucansucrase from *Lactobacillus* strain LB 33.**

A new *Lactobacillus* strain was obtained and was deposited as LMG P-20349. The strain was identified by 16S rRNA to be most closely related to *Lactobacillus parabuchneri*. The strain grown overnight on MRS supplemented with sucrose under anaerobic conditions at 37°C (see Example 2). 420 gram of glucan was produced. The glucan produced by this strain is not produced during growth on glucose.

Methylation analysis (see Example 2) revealed that the polymer consists of equal amounts of 29-39% of  $\alpha(1-3)$  linked glucosyl units, 30-40% of  $\alpha(1-6)$  linked glucosyl units, 3-13% of  $\alpha(1-3-6)$  linked glucosyl units (branching units) and 15-30% of 1-linked (terminal) glucose units.

The average molecular weight of the polysaccharide was established to be  $2 \times 10^5$  Da, using the SEC-MALLS system as described in Example 2.

By PCR with degenerated primers part of a sucrase type of glucosyl-transferase could be isolated indicating that the glucan is produced by a sucrase. This confirms the result that the glucan is produced during growth on sucrose and not on glucose. Part of the sucrase encoding gene was sequenced. On the deduced amino acid level high homologies were found with alternan sucrase from *Leuconostoc mesenteroides*. This indicates that the enzyme responsible for the glucan synthesis in *L. brevis* is the first alternan sucrase found in other bacteria than *Leuconostoc*. This partial sequence information is given in SEQ ID No. 3 (DNA) and 4 (protein). Full sequence information is given in SEQ ID No's. 17 and 18, respectively.

The glucan produced by this strain has thickening properties.

**Example 5: Isolation and identification of  $\alpha$ -(1,6) glucan and a glucansucrase from *Leuconostoc* strain 86**

A new strain was obtained from silage and was deposited as LMG P-20350. The strain was identified by 16S rRNA to be a new *Leuconostoc* strain, most closely related to  
5 *Leuconostoc citreum*. The strain grown overnight on MRS supplemented with sucrose under anaerobic conditions at 37°C (see Example 2). 416 gram of glucan was produced. Methylation analysis of the glucan obtained revealed that more than 90 % of the glucose units was linked through an  $\alpha$  (1,6) bond, identifying the polysaccharide as a dextran. The molecular weight of the glucan (determined as described in Example 2) was  $3-4 \times 10^7$  Da  
10 and the Rg was 40 nm. The glucan is not produced during growth on glucose.

By PCR with degenerated primers 3 different fragments with part of a sucrase type of glucosyl-transferase could be isolated indicating that the glucan is produced by a sucrase and that possibly 3 sucrases are present in this strain. This confirms the result that the glucan is produced during growth on sucrose and not on glucose. Part of the sucrase  
15 encoding gene was sequenced. On the deduced amino acid level high homologies were found with DSRC and DSRB (fragment 1), alternan sucrase (fragment 2) and DSRA (fragment 3) from *Leuconostoc mesenteroides*. The sequence information is given in SEQ ID No's 5-10. *Leuconostoc citreum*, to which this new strain is most closely related, is not reported to produce dextran. The glucan produced by strain 86 has thickening properties.  
20

**Example 6: Identification of  $\alpha$ -(1,6/1,3) glucan and a glucansucrase from *Lactobacillus sake* KG 15**

Strain KG 15 was obtained from silage and was deposited as LMG P-21583. It was identified by 16S rRNA as *L. sake*. The strain was grown and the polysaccharide was  
25 recovered as described in example 2. The molecular weight of the polysaccharide was determined to be  $4,7 \cdot 10^7$  Da (SEC MALLS) and the Rg was 92 nm. Methylation analysis (GC) revealed that the glucan produced by this strain is a largely linear dextran containing 4 % terminal glucose units, 86% of  $\alpha$ (1,6) linked glucosyl units, 2% of  $\alpha$  (1,3) linked glucosyl units and 8%  $\alpha$  (1,3,6) disubstituted glucose units (branching points). The  
30 glucansucrase of this strain was sequenced (see SEQ ID No. 19 and 20).

**Example 7: Identification of  $\alpha$ -(1,6/1,3) glucan and a glucansucrase from *Lactobacillus fermentum* KG 3**

Strain KG 3 was obtained from silage and was deposited as LMG P-21584. It was  
35 identified by 16S rRNA as *L. fermentum*. The strain was grown and the polysaccharide was recovered as described in example 2. The molecular weight of the polysaccharide was determined to be  $2,4 \cdot 10^7$  Da (SEC MALLS) and the Rg was 107-119 nm. Methylation analysis (GC) revealed that the glucan produced by this strain is a largely linear dextran containing 3% terminal glucose units, 84% of  $\alpha$ (1,6) linked glucosyl units,

8% of  $\alpha$  (1,3) linked glucosyl units and 5%  $\alpha$  (1,3,6) disubstituted glucose units (branching points). The glucansucrase of this strain was sequenced (SEQ ID No's 21 and 22).

5 **Example 8: Anticorrosion properties of glucans**

Plain carbon steel sheets of 1 cm<sup>2</sup> embedded in an epoxy matrix were exposed to a slightly corrosive medium (150 ml of 0.1 M LiClO<sub>4</sub>) with or without the addition of a bacterial polysaccharide (0.2 g) for several days. The sheets were then examined visually and electrochemically from time to time. The corrosion potential ( $E_{\text{corr}}$  in mV with reference to Ag/AgCl) and polarisation resistance ( $R_p$  in k $\Omega$ /cm<sup>2</sup>) are both a measure of the anti-corrosion effect. After an initial adaptation of 3-10 hours, these parameters attained a stable value. The experiments were carried with a heteropolysaccharide from *Lactobacillus sake*, and a homopolysaccharide of the invention (from LB 180 according to example 4), as well as without polysaccharide. The results are summarised in the table below. It follows that the anti-corrosion properties of the glucan of the invention are superior. It was found that the homopolysaccharide of ML 1 (example 3) has at least equal anticorrosion performance as the LB 180 polysaccharide.

*Table: Corrosion experiments*

organism	type of polysaccharide	aspect of treated sheet	$E_{\text{corr}}$ (mV vs. Ag/AgCl)	$R_p$ (k $\Omega$ /cm <sup>2</sup> )
control	-	corrosion	-700	1.5
<i>Lb. sake</i>	heteropolysaccharide	localised corrosion	-600	4.5
<i>Lb. 180</i>	$\alpha$ -glucan	thin black layer	-200	70

**Example 9: Modification of  $\alpha$ -1,3/1,6-glucan by oxidation**

One gram (6.15 mmol of anhydroglucose units) of the  $\alpha$ -1,3/1,6-glucan produced by strain LB 33 (example 4) is resuspended in 100 ml water. Next, 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO; 0.01 g, 0.065 mmol) and sodium bromide (100 mg, 1 mmol) are added and the suspension is cooled to 0°C. The reaction can also be performed without bromide. A solution of hypochlorite (3 ml, 15% solution, 6.3 mmol) of pH 10.0 (0°C) is added. The pH is kept constant by addition of 0.1M NaOH. After 1 hr, the solution is poured into 150 ml 96% ethanol, causing the product to precipitate. The white precipitate is centrifuged, resuspended in ethanol/water (70/30 v/v) and centrifuged again. Next, the precipitate is resuspended in 96% ethanol, centrifuged and dried. The uronic acid content is determined by means of the uronic acid assay according to Blumenkrantz and Abdoe-Hansen (*Anal. Biochem.* 54 (1973), 484). A calibration curve was generated using polygalacturonic acid (5, 10, 15 and 20  $\mu$ g). With this calibration curve the uronic

acid content in a sample of 20  $\mu$ g of the product is determined. The major part of 6-hydroxyl groups have been oxidised to carboxyl groups.

**Example 10: Construction of plasmids for expression of the glucansucrase genes in *E. coli*.**

- 5 Two primers were designed with appropriate restriction sites; the C-terminal primer contained in all cases a His-tag. The PCR products were first cloned in pCR-XL-TOPO. The PCR products were removed from pCR-XL-TOPO using the appropriate enzymes and ligated in the appropriate sites of an expression vector (e.g pET15b (Novagen) ).
- 10 For the expression of part of the glucosyltransferase gene of LB 180 (for better expression, the N-terminal region encoding the N-terminal variable domain of the glucansucrase, was not cloned) in *E. coli*, a PCR reaction was performed using Forw180 ( 5'-GATGCATGAG **CTCCCATGGG** CATTAAACGGC CAACAATATT ATTATTGACC C-3') containing *SacI* (bold) and *NcoI* (underlined) sites, and Rev180 (5'-ATATCGATGG GCCCCGGATC CTATTAGTGA *TGGTGATGGT* GATGTTTTTG
- 15 GCGTTTAAA TCACCAGGTT TTAATGG-3'), containing *ApaI* (bold), *BamHI* (underlined) and a 6x His-tag (*italics*) as primers. The PCR product was cloned in pCR-XL-TOPO. The PCR product was removed from pCR-XL-TOPO using *NcoI/BamHI* and ligated in the corresponding sites of pET15b (Novagen). The resulting plasmid (pET15b180) containing part of the glucansucrase gene of 704 amino acids encoding a
- 20 glucansucrase without the variable N-terminal domain was transformed to *E. coli* B121 DE3 star (Invitrogen).

Cells of *E. coli* harbouring the pET15b180 were harvested by centrifugation after 16 h of growth under aerobic conditions at 37 °C. The pellet was washed with 50 mM sodium acetate buffer pH 5.5 containing 1 mM CaCl<sub>2</sub> and 1% (v/v) Tween 80 and the suspension

25 was centrifuged again. Pelleted cells were resuspended in with 50 mM sodium acetate buffer pH 5.5 containing 1 mM CaCl<sub>2</sub> and 1% (v/v) Tween 80, and 7.2 mM  $\beta$ -mercaptoethanol. Cells were broken by sonication and cell debris and intact cells were removed by centrifugation for 15 minutes at 4 °C at 14,000 rpm (Eppendorf). The resulting cell free extract was used as enzyme source to produce high molecular weight glucans from

30 sucrose in 50 mM sodium acetate buffer pH 5.5 containing 1 mM CaCl<sub>2</sub> and 1% (v/v) Tween 80 and 10 g/l sucrose. After 16 hours of incubation, the glucans were isolated using ethanol precipitation. When cell free extracts of *E. coli* B121 DE3 star (Invitrogen) harbouring the plasmid pET15b (without insert) were used as enzyme source, no glucans were produced from sucrose.

35

#### Sequence information

SEQ ID No's 1 and 2 give the nucleotide and amino acid sequence, respectively, of a part of the glucansucrase from strain Lb180 as originally determined (Example 2). The partial

sequence shows 53% (199/223) sequence identity and 68% similarity with dextransucrase DSRB742 of *Leuconostoc mesenteroides* (*Lc. mes.*), with 2 gaps (between amino acids F172 and N173), and 52% identity with some other dextransucrases and alternansucrases of *Lc. mes.*

5 SEQ ID No's 3 and 4 give the nucleotide and amino acid sequence, respectively, of a part of the glucansucrase from strain Lb 33 as originally determined (Example 4). The partial sequence shows 63% (143/224) sequence identity and 75% similarity with dextransucrase DSRB742 of *Lc. mes.* with 1 gap.

10 SEQ ID No's 5 and 6 give the nucleotide and amino acid sequence, respectively, of a part of a glucansucrase (86-1) from strain Lc 86 (Example 5). The partial sequence shows 98% (219/223) sequence identity and 99% similarity with dextransucrase DSRB742 of *Lc. mes.*

15 SEQ ID No's 7 and 8 give the nucleotide and amino acid sequence, respectively, of a part of another glucansucrase (86-5) from strain Lc 86 (Example 5). The partial sequence shows 55% (123/223) sequence identity and 68% similarity with dextransucrase DSRB742 of *Lc. mes.*, with 2 gaps (between amino acids M128 and R129 and between D162 and H163), and 51-56% identity with some other dextransucrases and alternansucrases of *Lc. mes.*

20 SEQ ID No's 9 and 10 give the nucleotide and amino acid sequence, respectively, of another glucansucrase (86-8) from strain Lc 86 (Example 5). The partial sequence shows 61-68% sequence identity and 74-78% similarity with dextransucrases and alternansucrases (including dextransucrase DSRB742) of *Lc. mes.*

25 SEQ ID No's 11 and 12 give the nucleotide and amino acid sequence, respectively, of the glucansucrase of strain Lb180 (Example 2). The sequence shows 1322/1768 (74%) sequence identity and 1476/1768 (82%) similarity with 15/1768 gaps with glucansucrase from *Lb. reuteri* LB 121 as disclosed in WO 01/90372. The -35 and -10 sites TTGAAA and TATAA are located at nucleotide positions 561 and 599, respectively. The ribosome binding site (RBS) GAAGGAG is at 574 and the start codon ATG at 587. Inverted repeats AAGCAGCTC and GAGCTGCTT are at 6025 and 6051. Possible stop codons (TAA, TAG, TGA) are indicated with an \* (5963).

30 SEQ ID No's 13 and 14 give the nucleotide and amino acid sequence, respectively, of the glucansucrase I from strain ML1 (Example 3). The sequence shows 1327/1775 (74%) sequence identity and 1465/1775 (81%) similarity with 17/1775 gaps with glucansucrase from *Lb. reuteri* LB 121 as disclosed in WO 01/90372, and 43-44% sequence identity and 57-58% similarity with dextransucrases of *Lc. mes.* and 47% sequence identity and 61% similarity with an alternansucrases of *Lc. mes.* The RBS AAGGAGA is at 31 and the start codon ATG is at 43. A stop codon TAG is at 5356.

35 SEQ ID No's 15 and 16 give the partial nucleotide and amino acid sequence, respectively, of a second glucansucrase from strain ML1 (ML4) (Example 3). The sequence shows

301/817 (36%) sequence identity and 427/817 (51%) similarity with 12/817 gaps with glucansucrase from *Lb. reuteri* LB 121 as disclosed in WO 01/90372, and 38% sequence identity and 53% similarity with glucosyltransferase of *Streptococcus mutans*.

5 SEQ ID No's 17 and 18 give the partial nucleotide and amino acid sequence, respectively, of the glucansucrase from strain LB 33 (Example 4). The sequence shows 59% sequence identity and 71% similarity with several known dextranases of *Lc. mes.* and 53% sequence identity and 67% similarity with other known dextranases (including dextranase DSRB742) of *Lc. mes.*

10 SEQ ID No's 19 and 20 give the nucleotide and amino acid sequence, respectively, of the glucansucrase from *Lb.* strain KG 15 (Example 6). The sequence shows 496/1111 (44%) sequence identity and 637/1111 (56%) similarity with 71/1111 gaps with glucansucrase from *Lb. reuteri* LB 121 as disclosed in WO 01/90372, and 57-59% sequence identity and 70% similarity with several dextranases (including dextranase DSRB742) of *Lc. mes.* The -35 and -10 sites *TTGGAC* and *TATTAT* are located at nucleotide positions 477 and 502, respectively. The RBS *GAAAGGA* is at 593 and the start codon *ATG* at 608. A stop codon *TAG* is 5393. Inverted repeats *AAAACAACCCCC* and *GGGGTTGTTTTT* are at 5497 and 5531 (-10.7 kcal/mole).

15 SEQ ID No's 21 and 22 give the partial nucleotide and amino acid sequence, respectively, of the glucansucrase from *Lb.* strain KG 3 (Example 7). The sequence shows 58 sequence identity and 71% similarity with known dextranases (including dextranase DSRB742) of *Lc. mes.*

### Description of the figure

25 Figure 1 depicts an amino acid sequence alignment of glucosyltransferases (GTF) according to the invention. It shows the partial sequences of the GTF of *Lb* 180 (first line, starting with amino acid 216 of SEQ ID No. 12); GTF of ML1 (second line, starting with amino acid 15 of SEQ ID No. 14), GTF of *Lb* 33 (third line, starting with amino acid 222 or 243 of SEQ ID No. 18); GTF of KG15 (fourth line, starting with amino acid 567 of SEQ ID No. 20) and GTF of KG3 (fifth line, starting with amino acid 1 (LMAAF) of SEQ ID No. 22); and a GTF according to the invention of a *Lb. reuteri* strain "104" (sixth line, 1 (WPNTV) - 525). The alignment is not necessarily the best fit according to automated alignment programs, but is intended to define the enzymes of the invention.

35 The invention not only covers amino acid sequences shown in this figure, but also sequences wherein amino acids of a given sequence in the figure are exchanged with the corresponding amino acids (including gaps) of another sequence of the figure. This applies to stretches of at least 100 amino acids having at least 80%, preferably at least 90% identity with any of the sequences of the figure, or of the sequences listings given separately. It especially applies to the stretch of amino acids between the consensus peptides DNSN and YYGD (from 1202 to 1422 of SEQ ID No 12). Especially preferred



are sequences comprising the active core of the enzymes, which are present between the consensus peptides INGQ and VPDQ (from 957 to 1724 of SEQ ID No 12), with preferably at least 70% identity with any one of the core sequences given. A preferred non-identity with a given sequence is an exchange with the corresponding amino acids of another sequence. Especially preferred sequences are those where an amino acid at a given position is shared between at least 2, in particular at least 3, of the sequences of the figure. Most preferred are those sequences in which one of those consensus sequences is that of the GTF of Lb180, ML1 or Lb33 (first three lines). The N-terminal part upstream of the core (shown in the figure for GTF 180 and GTF ML1 only), or the C-terminal part downstream of the core (not shown in the figure) may be wholly or partly present or may be absent.

**Claims**

1. A process of producing a glucan having at least 10 anhydroglucose units, having a backbone consisting essentially of  $\alpha(1,3)$ - and/or  $\alpha(1,6)$ -linked anhydroglucose units (AGU), comprising subjecting sucrose to the activity of a glucosyltransferase produced by a *Lactobacillus* strain capable of producing  $\alpha(1,3)$ - and/or  $\alpha(1,6)$ -linked glucans, or to the *Lactobacillus* strain capable of expressing the glucosyltransferase.
2. A *Lactobacillus* strain capable of producing, in the presence of sucrose, a glucan having at least 10 anhydroglucose units (AGU) having a backbone consisting essentially of  $\alpha(1,3)$ - and/or  $\alpha(1,6)$ -linked AGU.
3. A glucan capable of being produced by glucosyltransferase activity of a lactic acid bacterium on a sucrose substrate, the glucan having an average molecular weight between 10 kDa and 1 GDa, especially between 10kDa and 50 MDa, and having a backbone consisting essentially of  $\alpha(1,3)$ - and  $\alpha(1,6)$ -linked anhydroglucose units (AGU).
4. A glucan according to claim 3, which is capable of being produced by glucosyltransferase activity of a *Lactobacillus* species.
5. A glucan according to claim 4, comprising 15-80% of  $\alpha(1,3)$ -linked AGU, 2-80% of  $\alpha(1,6)$ -linked AGU, and 2-25% of  $\alpha(1,3,6)$ -linked AGU.
6. A glucan according to claim 5, having an average molecular weight of 50 kDa - 1 MDa and comprising 30-45% of  $\alpha(1,3)$ -linked AGU, 30-45% of  $\alpha(1,6)$ -linked AGU, and 3-13% of  $\alpha(1,3,6)$ -linked AGU.
7. A glucan according to claim 5, having an average molecular weight of 10-50 MDa and comprising 15-26%  $\alpha(1,3)$ -linked AGU, 30-50% of  $\alpha(1,6)$ -linked AGU, 5-20% of  $\alpha(1,3,6)$ -linked AGU.
8. A glucan according to claim 5, having an average molecular weight of 1-50 MDa and comprising 45-60% of  $\alpha(1,3)$ -linked AGU, 4-10% of  $\alpha(1,6)$ -linked AGU, and 10-20% of  $\alpha(1,3,6)$ -linked AGU.

9. A glucan capable of being produced by glucosyltransferase activity of a lactic acid bacterium on a sucrose substrate, having an average molecular weight of 10-50 MDa and comprising 80-99% of  $\alpha(1,6)$ -linked AGU and 0-15% of  $\alpha(1,3)$ -linked AGU.
10. A protein having glucosyltransferase activity, capable of producing, in the presence of sucrose, a glucan according to any one of claims 3-9.
11. A protein according to claim 10, comprising an amino acid sequence of at least 100 amino acids exhibiting at least 70%, preferably at least 80%, amino acid identity with any one of the amino acid sequences of SEQ ID No. 2, 4, 8, 10, 12, 14, 16, 18, 20 and 22, and/or having a stretch of 100 amino acids having at least 80%, preferably at least 90%, amino acid identity with any one of the said amino acid sequences, or having at least 99% amino acid identity with the amino acid sequence of SEQ ID No. 6, and/or having a stretch of 100 amino acids having 100% amino acid identity with the amino acid sequence of SEQ ID No. 6.
12. A nucleic acid sequence encoding a protein according to claim 11.
13. A recombinant host cell containing one or more copies of a nucleic acid construct comprising a nucleic acid sequence according to claim 12 and capable of expressing a protein having glucosyl-transferase activity.
14. A *Lactobacillus* strain, capable of producing a glucan according to any one of claims 3-9, especially a *Lactobacillus* strain corresponding to strain 33, 180 or ML1 as described herein.
15. A *Leuconostoc* strain, capable of producing a glucan according to claim 9, especially a *Leuconostoc* strain corresponding to strain 86, deposited under accession number LMG P-20350.
16. A chemically modified glucan, which is obtained by 2,3-oxidation, 6-oxidation, phosphorylation, acylation, alkylation, hydroxyalkylation, carboxymethylation, amino-alkylation of one or more AGU of a glucan according to any one of claims 3-9.
17. Use of a glucan according to any one of claims 3-9, as a thickener.
18. Use of a glucan according to any one of claims 3-9, as a prebiotic and/or as a bioactive agent.

19. Use of a glucan according to any one of claims 3-9, as an anti-corrosion agent.
20. Use of a *Lactobacillus* bacterium capable of producing a glucan according to any one of claims 3-9, as a probiotic agent, or together with an indigestible glucan, as a synbiotic agent.

**FIG. 1 SEQUENCE ALIGNMENT**

216 MEIKKHFKLYKSGKQWVTA AVATVAVSTALLYGGVAHADQQVQSSTTQEQTSTVNADTTK  
 15 MEIKKHFKLYKSGKQWVTA AVATVAVSTALLYGGVAHADQQVQSSTTQDQTSTVNTNTTK  
  
 276 TVNLDTNTDQPAQTDDKNQVANDTTTNQSKTDSTSTTVKNPTFIPVSTLSSSDNEKQSQN  
 75 TIAADTNADQPAQTADKNQAASNDTTTNQSKTDSTSTTVKNLTSTFPVSTLPSTDNEKQSQN  
  
 336 YNKPDNGNYGNVDAAYFNNNQLHISGWHATNASQGTDSRQVIVRDITTKTELGRNTVNTNN  
 135 YNKHDNGNYGNIDTAYFSNNQLHVS GWNATNASQGTNSRQIIVRDITTNNELGRTDVNTNN  
  
 396 VLRPDVKNVHN VYNADNSGFDVNINIDFSKMKDYRDSIEIVSRYSNGNGKSVDWWSQPITF  
 195 VARPDVKNVHN VYNADNSGFDINVNIEFSKMKDYRDSIEIVSRYSNGNGKSIDWWSQPITF  
  
 456 DKNNYAYLDTFEVKNGELHATGWNATNKAINYNHHFVILFDRTNGKEVTRQEV RDGQSRP  
 255 DKNNYAYLDTFEVKNGELHATGWNATNSAINYNHHFVILFDQTNNGKEVARQEVREGQSRP  
  
 516 DVAKVYPQVVGANN SGFDVTFNIGDL DYTHQYQILSRYSNADNGEGDYV TYWFAPQSIAP  
 315 DVAKVYPQVVGADNSGFDVTFNIGNLDYTHQYQVLSRYSNSDNGEGDNV TYWFPQSIAP  
  
 576 ANQSNQGYLDSFDISKNGEVTVTGWNATDLSELQTNHYVILFDQTAGQQVASAKVDLISR  
 375 ANQSNQGYLDSFDISKNGEVTVTGWNATDLSELQNNHYVILFDQTAGKQVASAKADLISR  
  
 636 PDVAKAYPTVKTAETSGFKVTFKVSNLQPGHQYSVVS RFSADENGNGNDRHTDYWYSPV  
 435 PDVAKAYPTVKTAANS GFKVTFKVN DLQPGHQYSVVS RFSADENGNGNDRHTDYWYSPV  
  
 696 TLNQ TASNIDTITMTS NGLHITGWMASDNSINEATPYAIILNNGREVTRQKLT LIARPDV  
 495 TLNQ NASNIDTITMTS NGLHIGSWMASDNSINETTPYAIILNNGKEVTRQKMSLTARPDV  
  
 756 AAVYPSLYNSAVSGFDTTIKLTNAQYQALNGQLQVLLRFSKAVDGNPNGTNTVTDQFSKN  
 555 AAVYPSLYNSAVSGFDTTIKLTNDQYQALNGQLQVLLRFSKAADGNPSGDNTVTDQFSKN  
  
 816 YATTGGNFYDVKVNGNQIEFSGWHATNQSN DKNSQWIIVLVNGKEVKRQLVNDTKDGAAG  
 615 YATTGGNFYDVKVNGNQVEFSGWHATNQSN DKDSQWIIVLVNGKEVKRQLVNDTKEGAAG  
  
 876 FNRNDVYKVNPAIENSIMSGFQGIITLPVTVKDEN VQLVHRFSNDAKTGEGNYVDFWSEV  
 675 FNRNDVYKVNPAIENS SMSGFQGIITLPVTVKNE NVQIVHRFSNDAKTGEGSHVDFWSEV  
  
 936 MSVKDSFQKGNGLNQFGLQTINGQYYIDPTTGQPRKNFLLQNGNDWIYFDKDTGAGTN  
 735 MPVKDSFQKGNGLKQFGLQTINGHQYYIDPMTGQPRKNFLLQNGNDWLYFDNETGEGTN  
 222 VNGKIYFVGDNQVKKNF TAIINGQSLYFNKTTGELASNDVQYENGLVKINDV  
 567 QTIAGKTY YFDKD GHLRKGYSTIIDNQLYYFDLKTGESVS  
  
 996 ALKLQFDKGTISADEQYRRGNEAYS YDDKSIENVNGYLTADTWYRPKQILKDGT TWTDSK  
 795 ALKRQFDGGTISADSQYRK GNEAYGDNKSIENVDGFLTADTWYRPKQILKW TWTDSK  
 275 HNAAYSIDP?GFTNVNGFLTANSWYRPKYIYKDGQKQWVEST  
 607 TTTSNFKSGLTSQTDDTTPHNSAVNMSKDSFTTVDGFLTAE SWYVPKDIQTSATDWRAS T  
  
 1056 ETDMPILMVWWPNTVTQAYYLN YMKQYGNLLPASLP SFST DADS AELNHYSELVQQNIE  
 854 ETDMPRLLMVWWPNTVTQAYYLN YMKQHGNLLPANLPFFNSDADPLELNYYAEIVQQNIE  
 316 SQDMPRLLMTW PDKNTQVAYLQYM QKMGI LPADV TISSQTNQSVLTKE SFITQAEIE  
 666 PEDFRPIMMTW WP TKQIQ AAYLNH MVSEG LLSSDKKFSATD DQTL LNQA AHAVQLQIE  
 (0)  
 1 WPNTVTQAYYLN YMKQHGNLLPASLPFFNADADPAELNHYSEIVQQNIE

1116 KRISSET GSTDWLRTLMEHFVTKNSMWNKDSENVYGGGLQLQGGFLKYVNSDLTKYANSW  
914 KKISQT GNTDWLRTLMEHFVSNNTMWNKSENEDEFGGLQLQGGFLKYVNSDKTPNANSNW  
374 KQIGVTNGNTDWLKKDISDFVNSQPNWNIDSEAKGTDH LQGGALLYVNNKLTYPYANSY  
725 LKIQQT KSVEWLRTTMHNFIKSQPGYNVTSETPSNDH LQGGALSYINSVLTDPDANSNF  
1 LMAAFVVTQPQWNKTSEDVNDDH LQGGALT FENNGDT DANDSY  
50 KRISSET GNTDWLRTLMEHFVTKNSMWNKDSENVYGGGLQLQGGFLKYVNSDLTKYANSW

1176 RLMNRTATNIDGKNY GGAEFLLANDIDNSNPVVQAEELNWLYYLMNFGTITGN  
974 RIMGRQPANIDGNP IGSEFLLANDVDNSNPVVQAEQLNWLHYLLNFGTITAN  
433 RLLNRTLNTNQQGQVKDTS KQGGYEMLLANDVDNSNPVVQAEQLNWLYYMMNIGSITAN  
783 RLMNRNPTQQDGTRHYNTDTSSEGGYELLLANDVDNSNPVVQAEQLNWLHYFLTHFGEIVKN  
44 RLMNRTPTNQTGERLYHIDDSLGGYELLLANDVDNSNPQVQAEQLNWLHYLMHFGDITAD  
110 RLMDRATATNIDGKNY GGAEFLLANDIDNSNPVVQAEELNWLYYLMNFGTITGN

1229 NPEANFDGIRVDAVDNVDVLLSIARDYFNAAYNMEQSDASANKHINILEDWGWD DPAYV  
1027 DPDANFDSIRVDAVDNVDADLLDIAGDYFNAVYHSQSNDKIANAHINILEDWGQDPYYT  
491 DPTANFDGYRVDVDNVDADLLNIAADYAKAYKTN QSDANANKHLSILEDWDNDNDPAYI  
843 DPSANFDSVRVDVDNVDADLLNITAAFRDVGVDKNDLTANQHLSILEDWGHNDDPLYV  
104 DPDANFDAIRIDVDNVDADLLQLAAQYFRDAYGMATTDATSNKHLSILEDWSHNDPAYM  
163 NPEANFDGIRVDAVDNVDVLLSIARDYFNAAYNMEQSDANANKHINILEDWGWD DPAYV

1289 NKIGNPQLTMDDRLRNAIMDTLSGAPDKNQALNKLITQSLVNRANDN TENAVIPSYNFV  
1087 QSIGTPQLSMDYNFSTIRSVLASNTASMTD IIKNSLVNRSLDN AENVSI PNYSFI  
551 KAHGNNQLTMDFPAHLAIKYSLNMPVSQSRGLEPELTTSLVNRTGDDSTENVAQPNYTFI  
903 KDHGSDQLTMDDYMHTQLIWSLTKNPDNRSAMRRFMEYYLVDRADN TSDPAIPNYSEFV  
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223 NKIGNPQLTMDDRLRNAIMDTLSGAPDKNQALNKLITQSLVNRANDN TENAVIPSYNFV

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611 RAHDSEVQTIIAQIIKDKINPNSDGLTVPDEISQAFKIYNADELKTDKQYTFYNMPSAY  
962 RAHDSEVQTVIGDIVAKLYPDVKNSL PSMEQLAAAFKVYDADMNSVNKKYTQYNMPAAY  
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1021 AMLLTNKDTPRVYYGDMYTDGQYMATKSPYYDAISALLKARIKYVAGGQTM MAVDKH  
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1464 GILTNVRFKGKAMNATDTGTDETRTEGIGVVISNNTNLKLN DGESVVLHMG  
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392 LAHANQAFRAVLLTTATGLTIY NDDDAPIRYTDNKGDLIFTNHDV YG  
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1177 YQNVEVSGFLSVWVPVGASDTQDARATGSSAANKTGDTLHSNAALDSNVIYEGFSNFQEM  
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505 YANPDVTGYLAVWVPAGAADD

1631 PTTESERTNVRIAQNADLFKSWGITT FELAPQYNSSKDGTFLDSIIDNGYAFTDRYDLGM  
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1237 PTAHDEFTNVKIAQNADLFKSWGVTSEQLAPQYRSSDDTSFLDSIIKNGYAFTDRYDLGF  
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lactic acid bacteria

&lt;130&gt; Novel glucans and glucansucrases

&lt;140&gt;

&lt;141&gt;

&lt;160&gt; 10

&lt;170&gt; PatentIn Ver. 2.1

&lt;210&gt; 1

&lt;211&gt; 665

&lt;212&gt; DNA

&lt;213&gt; Lactobacillus reuteri

&lt;400&gt; 1

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&lt;210&gt; 2

&lt;211&gt; 221

&lt;212&gt; PRT

&lt;213&gt; Lactobacillus reuteri

&lt;400&gt; 2

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      20             25             30
Phe Asp Gly Ile Arg Val Asp Ala Val Asp Asn Val Asp Val Asp Leu
      35             40             45
Leu Ser Ile Ala Arg Asp Tyr Phe Asn Ala Ala Tyr Asn Met Glu Gln
      50             55             60
Ser Asp Ala Ser Ala Asn Lys His Ile Asn Ile Leu Glu Asp Trp Gly
      65             70             75             80
Trp Asp Asp Pro Ala Tyr Val Asn Lys Ile Gly Asn Pro Gln Leu Thr
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 Phe Val Arg Ala His Asp Ser Asn Ala Gln Asp Gln Ile Arg Gln Ala  
 145 150 155 160  
 Ile Gln Ala Ala Thr Gly Lys Pro Tyr Gly Glu Phe Asn Leu Asp Asp  
 165 170 175  
 Glu Lys Lys Gly Met Glu Ala Tyr Ile Asn Asp Gln Asn Ser Thr Asn  
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 Lys Lys Trp Asn Leu Tyr Asn Met Pro Ser Ala Tyr Thr Ile Leu Leu  
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 gtggacaatg tcgatgctga tttattaaat atagctgccg attatgcaa agatgcttat 180  
 aaaactaatc aaagtgatgc taatgccaac aaacatttat caatattaga agattgggat 240  
 aataatgac cggcttatat caaagcacat ggaaataatc agttaactat ggatttccca 300  
 gcacatttag caattaaata ttcattaaat atgccagtaa gtcaacgaag tgggctggaa 360  
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 agtcaggcct ttaaaatata taatgcagat gaattaaaga ctgataaaca atatactttt 600  
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 Phe Asp Gly Tyr Arg Val Asp Ala Val Asp Asn Val Asp Ala Asp Leu  
 35 40 45  
 Leu Asn Ile Ala Ala Asp Tyr Ala Lys Asp Ala Tyr Lys Thr Asn Gln

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50                      55                      60  
 Ser Asp Ala Asn Ala Asn Lys His Leu Ser Ile Leu Glu Asp Trp Asp  
 65                      70                      75                      80  
 Asn Asn Asp Pro Ala Tyr Ile Lys Ala His Gly Asn Asn Gln Leu Thr  
 85                      90                      95  
 Met Asp Phe Pro Ala His Leu Ala Ile Lys Tyr Ser Leu Asn Met Pro  
 100                      105                      110  
 Val Ser Gln Arg Ser Gly Leu Glu Pro Glu Leu Thr Thr Ser Leu Val  
 115                      120                      125  
 Asn Arg Thr Gly Asp Asp Ser Thr Glu Asn Val Ala Gln Pro Asn Tyr  
 130                      135                      140  
 Thr Phe Ile Arg Ala His Asp Ser Glu Val Gln Thr Ile Ile Ala Gln  
 145                      150                      155                      160  
 Ile Ile Lys Asp Lys Ile Asn Pro Asn Ser Asp Gly Leu Thr Val Thr  
 165                      170                      175  
 Pro Asp Glu Ile Ser Gln Ala Phe Lys Ile Tyr Asn Ala Asp Glu Leu  
 180                      185                      190  
 Lys Thr Asp Lys Gln Tyr Thr Phe Tyr Asn Met Pro Ser Ala Tyr Thr  
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 atttctgagt tacatcccga cgtaaaaaat agtttggcac caacagcaga ccagctagcc 540  
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 Phe Asp Glu Ile Arg Val Asp Ala Val Asp Asn Val Asp Ala Asp Leu  
 35 40 45  
 Leu Gln Ile Ala Ala Asp Tyr Phe Lys Ala Ala Tyr Gly Val Asp Lys  
 50 55 60  
 Asn Asp Ala Thr Ala Asn Gln His Leu Ser Ile Leu Glu Asp Trp Ser  
 65 70 75 80  
 His Asn Asp Pro Glu Tyr Val Lys Asp Phe Gly Asn Asn Gln Leu Thr  
 85 90 95  
 Met Asp Asp Tyr Met His Thr Gln Leu Ile Trp Ser Leu Thr Lys Asp  
 100 105 110  
 Met Arg Met Arg Gly Thr Met Gln Arg Phe Met Asp Tyr Tyr Leu Val  
 115 120 125  
 Asn Arg Asn His Asp Ser Thr Glu Asn Thr Ala Ile Pro Asn Tyr Ser  
 130 135 140  
 Phe Val Arg Ala His Asp Ser Glu Val Gln Thr Val Ile Ala Gln Ile  
 145 150 155 160  
 Ile Ser Glu Leu His Pro Asp Val Lys Asn Ser Leu Ala Pro Thr Ala  
 165 170 175  
 Asp Gln Leu Ala Glu Ala Phe Lys Val Tyr Asn Asn Asp Glu Lys Gln  
 180 185 190  
 Ala Asp Lys Lys Tyr Thr Gln Tyr Asn Met Pro Ser Ala Tyr Ala Met  
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&lt;211&gt; 746

&lt;212&gt; DNA

&lt;213&gt; Leuconostoc strain 86

&lt;400&gt; 7

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 aaggatggta aaatccttat tccctaattat agtttctgtac gtgcacacga taqtgaagtt 540

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caagggtatta ttggcaaata ttaacagatc atacgtcagc cgaatcaggt aataaattca 600  
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&lt;211&gt; 221

&lt;212&gt; PRT

&lt;213&gt; Leuconostoc strain 86

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Tyr Leu Met Asn Leu Gly Thr Ile Thr Ala Asn Asp Pro Asp Ala Asn  
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Phe Asp Ser Ile Arg Val Asp Ala Val Asp Asn Val Asp Ala Asp Leu  
 35 40 45

Leu Asp Ile Ala Arg Asp Tyr Phe Asn Ala Val Tyr Lys Val Asn Gln  
 50 55 60

Ser Asp Val Asn Ala Asn Lys His Ile Ser Ile Leu Glu Asp Trp Ser  
 65 70 75 80

Gly Leu Asp Pro Asn Glu Val Val Lys Asn Gly Asn Pro Gln Leu Thr  
 85 90 95

Leu Asn Thr Gly Val Gln Asn Ser Leu Leu Asn Ala Leu Thr Lys Gly  
 100 105 110

Pro Asn Asn Arg Trp Gly Ile Asp Ser Leu Ile Asp Lys Ser Thr Met  
 115 120 125

Arg Tyr Pro Asp Lys Asp Gly Lys Ile Leu Ile Pro Asn Tyr Ser Phe  
 130 135 140

Val Arg Ala His Asp Ser Glu Val Gln Gly Ile Ile Gly Lys Ile Leu  
 145 150 155 160

Thr Asp His Thr Ser Ala Glu Ser Gly Asn Lys Phe Thr Lys Asp Gln  
 165 170 175

Leu Lys Gln Ala Leu Asp Tyr Tyr Tyr Ala Asp Gln Asp Lys Thr Val  
 180 185 190

Lys Glu Tyr Ser His Tyr Asn Met Ala Ser Ala Tyr Ala Ala Leu Leu  
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Thr Asn Lys Asn Thr Ile Pro Asn Leu Tyr Tyr Gly Asp  
 210 215 220

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&lt;211&gt; 670

&lt;212&gt; DNA

&lt;213&gt; Leuconostoc strain 86

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&lt;400&gt; 9

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&lt;211&gt; 223

&lt;212&gt; PRT

&lt;213&gt; Leuconostoc strain 86

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 Phe Asp Gly Tyr Arg Val Asp Ala Val Asp Asn Val Asp Ala Asp Leu  
 35 40 45  
 Leu Gln Ile Ala Gly Asp Tyr Phe Lys Ala Ala Tyr Gly Thr Gly Lys  
 50 55 60  
 Thr Glu Ala Asn Ala Asn Asn His Ile Ser Ile Leu Glu Asp Trp Asp  
 65 70 75 80  
 Asn Asn Asp Ser Ala Tyr Ile Lys Ala His Gly Asn Asn Gln Leu Thr  
 85 90 95  
 Met Asp Phe Pro Ala His Leu Ala Leu Lys Tyr Ala Leu Asn Met Pro  
 100 105 110  
 Leu Ala Ala Gln Ser Gly Leu Glu Pro Leu Ile Asn Thr Ser Leu Val  
 115 120 125  
 Lys Arg Gly Lys Asp Ala Thr Glu Asn Glu Ala Gln Pro Asn Tyr Ala  
 130 135 140  
 Phe Ile Arg Ala His Asp Ser Glu Val Gln Thr Val Ile Ala Gln Ile  
 145 150 155 160  
 Ile Lys Asp Lys Ile Asn Thr Lys Ser Asp Gly Leu Thr Val Thr Pro  
 165 170 175  
 Asp Glu Ile Lys Gln Ala Phe Asn Ile Tyr Asn Ala Asp Glu Leu Lys  
 180 185 190  
 Ala Asp Lys Glu Tyr Thr Ala Tyr Asn Ile Pro Ala Ser Tyr Ala Val  
 195 200 205  
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210

215

220

SEQ ID No. 11 DNA

SEQ ID No. 12 PRT

Lactobacillus reuteri strain 180

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1 N S L P R S \* R I K T L K S L P L I L Q

61 GCATATCCAGCCAAAGCTTACCGTCTCGCCATTGAGTTATGAGTTTGAAGAAGGCAGAA  
21 H I Q P K L T V L A I Q L \* V \* R R Q K

121 GACACTGGGTTTGAGATTATGGATTGGCGGACTGCATTGAGTAAGTTTATAGAGGGGATT  
41 T L G L R L W I G G L H \* V S L \* R G L

181 GAGGAGTAAGATACTGGAACCGGTTTGGATTGGATACTGCTTTTTTATGGGCGGCGCAAT  
61 R S K I L E P V W I G Y C F F M G G A I

241 AAAGCTAGATCTAACTGGAAGAAAGACTGCGAACAAAATTGAAATTTAGTGTAAGCAGCTAA  
81 K L D L T G K D C E Q N \* N L V \* A A N

301 TATCCTTAGTCAATGTAGTATAATTGCAAATTTTTTACTAGGTAAGAAAGTATATTGTGG  
101 I L S Q C S I I A N F L L G K K V Y C G

361 AAATATTTAAGAATATTGTCGTTACCGGTAGAGACAATTTTATAAGTTCTAACTTTGTTC  
121 N I \* E Y C R Y R \* R Q F Y K F \* L C S

421 ACTATGTTGTTAACCCTTACTAGGAAGTTGAACATATTACGGTTTTAGATAAGTTAACTT  
141 L C C \* P L L G S \* T Y Y G F R \* V N L

481 ATACTGGCATTTAGTCAATTCTGATATCTTTGTTTAAAAATTACAAATTTGAACTTTGTTT  
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- 35

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181 K K M W E E F E N F L \* K N \* T S \* Y Y  
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601 TAATATCGATAATTAAATTGTTTATTCTGACATGAAGGAGATTAAAAATGGAATAAAGAA  
201 N I D N \* I V Y S D M K E I K M E I K K

661 ACATTTTAAGTTGTATAAAAAGTGGTAAACAATGGGTGACAGCGGCAGTTGCTACTGTTGC  
221 H F K L Y K S G K Q W V T A A V A T V A

721 CGTTTCAACCGCGCTTCTTTACGGGGGAGTTGCGCATGCTGATCAACAAGTTCACTCTTC  
241 V S T A L L Y G G V A H A D Q Q V Q S S

781 CACAACCCAAGAACAACTTCTACTGTGAATGCTGATACTACTAAAAACAGTAAATTTAGA  
261 T T Q E Q T S T V N A D T T K T V N L D

841 TACTAATACTGACCAACCAGCCCAAACAAGTATAAAAATCAAGTAGCAAATGACACTAC  
281 T N T D Q P A Q T T D K N Q V A N D T T

901 TACTAACCAAAAGTAAAACTGATAGTACATCAACAAGTGTAAAGAACCTACTTTTATACC  
301 T N Q S K T D S T S T T V K N P T F I P

961 AGTTTCTACTTTGTCTTCATCAGATAATGAAAAACAAAGTCAAAATTATAATAAGCCGGA  
321 V S T L S S S D N E K Q S Q N Y N K P D

1021 TAATGGAAACTATGGAAATGTTGATGCAGCTTACTTTAATAATAATCAATTGCATATTTTC  
341 N G N Y G N V D A A Y F N N N Q L H I S

1081 AGGATGGCACGCAACAATGCATCTCAAGGAACAGATAGTCGTCAGGTGATTGTACGTGA  
361 G W H A T N A S Q G T D S R Q V I V R D

1141 TATCACAACCTAAAACCTGAATTAGGACGTACTAATGTAACAAACAATGTTTTACGCCCAGA  
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1201 TGTTAAAAATGTCCACAATGTTTATAACGCTGATAATTCTGGATTTCGATGTCAACATCAA  
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1261 CATTGACTTTAGTAAGATGAAGGACTATCGTGATTCAATTGAAATTGTTAGTCGATACAG  
421 I D F S K M K D Y R D S I E I V S R Y S

1321 TGGAAATGGTAAATCTGTTGATTGGTGGTCTCAACCGATTACCTTTGACAAAAATAATTA  
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1381 CGCATACCTTGACACATTTGAAGTTAAAAATGGGGAATTGCATGCAACAGGATGGAATGC  
461 A Y L D T F E V K N G E L H A T G W N A

1441 TACTAATAAGGCAATTAACCTATAACCACCATTTTGTAAATTTTATTTGATCGAACAAATGG  
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1501 TAAAGAAGTGACTCGTCAAGAAGTTTCGTGATGGTCAATCGCGTCCAGATGTTGCTAAGGT  
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1561 ATATCCACAAGTAGTTGGGGCAAATAACTCTGGCTTTGACGTGACATTTAATATTGGTGA  
521 Y P Q V V G A N N S G F D V T F N I G D

1621 TCTAGATTACACTCATCAATACCAAATCTTAGTCGTTACAGCAATGCAGATAATGGCGA  
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1681 AGGTGATTATGTTACTTACTGGTTTGCTCCACAATCAATTGCTCCTGCTAACCAAAGTAA  
561 G D Y V T Y W F A P Q S I A P A N Q S N

1741 TCAGGGTTATTTAGATTCAATTTGATATTAGTAAAAATGGTGAAGTGACAGTAACTGGTTG  
581 Q G Y L D S F D I S K N G E V T V T G W

1801 GAATGCTACTGATCTATCTGAATTACAACTAACCATTATGTAATTTTATTTGACCAAAC  
601 N A T D L S E L Q T N H Y V I L F D Q T

1861 CGCTGGTCAACAAGTTGCATCTGCAAAAGTTGATCTAATTTCCCGTCCAGATGTTGCGAA  
621 A G Q Q V A S A K V D L I S R P D V A K

1921 AGCTTACCCAACAGTAAAAACTGCTGAAACTTCTGGCTTTAAGGTAAACATTTAAGGTTAG  
641 A Y P T V K T A E T S G F K V T F K V S

1981 TAATTTACAACCAGGTCATCAATATAGTGTGTAAGCCGTTTTTCTGCCGATGAAAACGG  
661 N L Q P G H Q Y S V V S R F S A D E N G

2041 TAATGGTAATGATAAACGTCATACCGATTACTGGTACAGCCCAGTAACCTTAAATCAAAC  
681 N G N D K R H T D Y W Y S P V T L N Q T

2101 TGCTTCAAATATTGATACTATCACAATGACATCGAATGGATTGCATATTACTGGTTGGAT  
701 A S N I D T I T M T S N G L H I T G W M

2161 GGCAAGTGATAATTCAATTAATGAAGCAACTCCATATGCCATTATTCTTAATAATGGTAG  
721 A S D N S I N E A T P Y A I I L N N G R

2221 AGAGGTTACTCGTCAAAAAATTAACCTTTAATTGCGCGTCCAGATGTAGCAGCAGTATATCC  
741 E V T R Q K L T L I A R P D V A A V Y P

2281 TTCACTCTATAACAGTGCTGTTAGTGGATTTGATACTACCATTAAAGTTGACTAATGCTCA  
761 S L Y N S A V S G F D T T I K L T N A Q

2341 ATACCAGGCGCTTAATGGTCAACTACAAGTATTGTTACGTTTTTCTAAAGCTGTTGATGG  
781 Y Q A L N G Q L Q V L L R F S K A V D G

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2401 TAATCCAAACGGCACTAATACTGTAACAGATCAATTTAGTAAGAATTATGCAACTACTGG  
801 N P N G T N T V T D Q F S K N Y A T T G

2461 TGGAAACTTTGATTATGTCAAAGTAAACGGCAATCAAATTGAATTTAGTGGCTGGCATGC  
821 G N F D Y V K V N G N Q I E F S G W H A

2521 AACTAATCAATCAAATGATAAAAATTCTCAATGGATTATTGTTTTAGTTAATGGTAAAGA  
841 T N Q S N D K N S Q W I I V L V N G K E

2581 GGTAACCGGCAATTAGTTAATGATACTAAGGATGGTGCTGCTGGGTTCAACCGTAATGA  
861 V K R Q L V N D T K D G A A G F N R N D

2641 TGTTTACAAAGTAAATCCGGCTATTGAAAATAGTATTATGTCTGGGTTCCAAGGTATTAT  
881 V Y K V N P A I E N S I M S G F Q G I I

2701 TACTTTACCTGTAACAGTTAAGGATGAAAATGTTTCAGCTTGTTTCATCGTTTTAGTAATGA  
901 T L P V T V K D E N V Q L V H R F S N D

2761 TGCAAAGACTGGTGAAGGTAATTATGTTGATTTCTGGTCAGAAGTAATGTCTGTTAAGGA  
921 A K T G E G N Y V D F W S E V M S V K D

2821 CAGCTTCCAAAAGGGTAATGGTCCGCTTAATCAATTTGGTTTACAACTATTAACGGCCA  
941 S F Q K G N G P L N Q F G L Q T I N G Q

2881 ACAATATTATATTGACCCAACAACCTGGCCAACCTCGTAAGAATTTCTTATTGCAAAATGG  
961 Q Y Y I D P T T G Q P R K N F L L Q N G

2941 GAACGATTGGATTTACTTTGACAAAGATACTGGTGCTGGAACATAATGCTCTTAAGTTACA  
981 N D W I Y F D K D T G A G T N A L K L Q

3001 ATTTGATAAGGGAACAATTTCTGCTGATGAGCAATATCGTCGAGGAAATGAAGCCTATAG  
1001 F D K G T I S A D E Q Y R R G N E A Y S

3061 TTATGATGACAAGAGTATTGAAAATGTAAATGGTTACTTAAACAGCTGATACTTGGTACCG  
1021 Y D D K S I E N V N G Y L T A D T W Y R

3121 ACCAAAACAAATCTTAAAGGATGGTACTACTTGGACTGACTCTAAAGAAACAGATATGCG  
1041 P K Q I L K D G T T W T D S K E T D M R

3181 CCCAATTTTAATGGTATGGTGGCCAAATACTGTTACACAAGCATATTATCTTAACTACAT  
1061 P I L M V W W P N T V T Q A Y Y L N Y M

3241 GAAGCAATATGGTAATTTATTGCCGGCTAGTTTACCAAGCTTCAGTACAGATGCTGATTC  
1081 K Q Y G N L L P A S L P S F S T D A D S

3301 TGCTGAATTAAATCATTACTCCGAGCTTGTTCAACAAAATATCGAAAAGCGGATCAGTGA  
1101 A E L N H Y S E L V Q Q N I E K R I S E

3361 GACTGGTAGTACTGATTGGTTACGTACACTAATGCATGAGTTCGTTACTAAGAATTCTAT  
1121 T G S T D W L R T L M H E F V T K N S M

3421 GTGGAATAAGGATAGTGAAAATGTCGATTACGGTGGTTTGCAATTACAAGGTGGATTCCCT  
1141 W N K D S E N V D Y G G L Q L Q G G F L

3481 TAAGTATGTAAATAGTGATCTTACTAAATATGCAAATTCAGATTGGCGTTTAAATGAACCG  
1161 K Y V N S D L T K Y A N S D W R L M N R

3541 TACAGCTACTAATATTGATGGTAAGAACTATGGTGGTGCGGAATTCTTATTAGCTAATGA  
1181 T A T N I D G K N Y G G A E F L L A N D

3601 TATTGATAACTCAAATCCAGTTGTTCAAGCTGAAGAATTAACTGGCTTTACTATTTAAT  
1201 I D N S N P V V Q A E E L N W L Y Y L M



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3661 GAATTTTCGGTACAATTACAGGAAATAATCCTGAAGCTAATTTTGATGGTATTCGAGTGG  
1221 N F G T I T G N N P E A N F D G I R V D

3721 TGCTGTTGATAATGTAGATGTTGACTTATTGAGTATTGCACGTGATTACTTTAATGCAGC  
1241 A V D N V D V D L L S I A R D Y F N A A

3781 ATATAACATGGAGCAAAGTGATGCCAGTGCTAATAAGCACATTAATATTTTGGAGATTG  
1261 Y N M E Q S D A S A N K H I N I L E D W

3841 GGGATGGGATGATCCTGCTTATGTAAATAAGATTGGAAATCCTCAATTAACAATGGATGA  
1281 G W D D P A Y V N K I G N P Q L T M D D

3901 TCGTTTACGAAATGCAATTATGGATACATTATCAGGAGCACCTGATAAAAACCAAGCATT  
1301 R L R N A I M D T L S G A P D K N Q A L

3961 GAATAAATTAATTACTCAGTCATTAGTAAATCGTGCTAATGATAATACTGAAAACGCGGT  
1321 N K L I T Q S L V N R A N D N T E N A V

4021 TATTCGAAGCTATAATTTTGTTCGAGCACATGATAGTAATGCTCAAGACCAAATTCGTCA  
1341 I P S Y N F V R A H D S N A Q D Q I R Q

4081 GGCTATTCAAGCTGCAACTGGAAAACCATATGGCGAATTTAACTTAGATGATGAAAAGAA  
1361 A I Q A A T G K P Y G E F N L D D E K K

4141 GGGTATGGAAGCATATATTAATGATCAGAATCTACTAATAAGAAGTGAATCTTTACAA  
1381 G M E A Y I N D Q N S T N K K W N L Y N

4201 TATGCCTTCTGCTTATACTATTCTTCTAACAATAAAGATTTCAGTTCCTCGTGTTTACTA  
1401 M P S A Y T I L L T N K D S V P R V Y Y

4261 TGGAGACCTCTACCAAGATGGTGGTCAATATATGGAACATAAAACACGTTACTTTGATAC  
1421 G D L Y Q D G G Q Y M E H K T R Y F D T

4321 TATTACTAACTTATTAAAGACACGGGTAAATATGTTGCCGGTGGACAAACTATGAGTGT  
1441 I T N L L K T R V K Y V A G G Q T M S V

4381 TGATAAGAATGGTATTCTTACAAACGTTCTGTTTTGGGAAAGGCGCCATGAATGCTACTGA  
1461 D K N G I L T N V R F G K G A M N A T D

4441 TACTGGTACTGATGAAACAAGAACAGAAGGTATCGGTGTTGTAATTAGTAACAATACTAA  
1481 T G T D E T R T E G I G V V I S N N T N

4501 TTTGAAGCTTAATGATGGTGAATCAGTAGTGCTTCATATGGGAGCTGCTCATAAGAATCA  
1501 L K L N D G E S V V L H M G A A H K N Q

4561 AAAGTATCGTGCTGTGATCTTAACAACCTGAAGATGGTGTTAAGAATTACACTAATGATAC  
1521 K Y R A V I L T T E D G V K N Y T N D T

4621 AGACGCACCAAGTTGCATACACTGATGCTAATGGTGACCTTCACTTTACTAATACTAATTT  
1541 D A P V A Y T D A N G D L H F T N T N L

4681 AGATGGTCAACAATATACAGCTGTTTCGTGGATATGCAAATCCTGATGTAACAGGATATCT  
1561 D G Q Q Y T A V R G Y A N P D V T G Y L

4741 AGCTGTTTGGGTACCAGCTGGAGCAGCAGATGATCAAGATGCACGTACTGCACCAAGTGA  
1581 A V W V P A G A A D D Q D A R T A P S D

4801 TGAGGCCCATACTACAAAGACTGCTTATCGCTCTAATGCAGCCCTTGATTCTAACGTTAT  
1601 E A H T T K T A Y R S N A A L D S N V I

4861 TTATGAAGGATTCTCTAACTTCATTTACTGGCCAACCTACTGAAAGCGAACGGACTAATGT  
1621 Y E G F S N F I Y W P T T E S E R T N V

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4921 GAGAATTGCACAAAATGCGGATCTATTTAAGTCATGGGGAATTACTACCTTTGAATTAGC  
1641 R I A Q N A D L F K S W G I T T F E L A

4981 TCCACAATACAATTCAAGTAAAGATGGTACGTTCCCTTGATTCAATAATTGATAATGGATA  
1661 P Q Y N S S K D G T F L D S I I D N G Y

5041 TGCCTTTACTGATCGTTATGATTTAGGAATGAGTACTCCTAACAAGTATGGATCTGATGA  
1681 A F T D R Y D L G M S T P N K Y G S D E

5101 AGACTTACGTAATGCTTTACAAGCCTTACATAAAGCTGGTTTACAAGCAATTGCCGACTG  
1701 D L R N A L Q A L H K A G L Q A I A D W

5161 GGTTCCTGATCAAATTTATAACTTACCTGGTAAAGAAGCTGTAACAGTAACACGTTTCAGA  
1721 V P D Q I Y N L P G K E A V T V T R S D

5221 TGATCACGGTACTACATGGGAAGTTTCGCCAATAAAGAATGTTGTCTATATTACAAATAC  
1741 D H G T T W E V S P I K N V V Y I T N T

5281 GATTGGTGGAGGTGAATACCAGAAGAAATATGGTGGTGAATTCTTAGACACTCTTCAAAA  
1761 I G G G E Y Q K K Y G G E F L D T L Q K

5341 AGAATATCCACAATTATTTAGTCAGGTATATCCAGTAACTCAAACGACAATTGATCCTAG  
1781 E Y P Q L F S Q V Y P V T Q T T I D P S

5401 TGTTAAGATTAAAGAGTGGTCTGCTAAATACTTTAATGGTACTAATATCCTTCATCGAGG  
1801 V K I K E W S A K Y F N G T N I L H R G

5461 TGCTGGATATGTATTGCGCTCTAATGATGGTAAATACTATAATCTTGGTACAAGCACTCA  
1821 A G Y V L R S N D G K Y Y N L G T S T Q

5521 ACAATTCTTACCGTCTCAATTATCAGTTCAAGATAATGAAGGATATGGATTTGTAAAAGA  
1841 Q F L P S Q L S V Q D N E G Y G F V K E

5581 AGGAAATAATTACCATTACTATGATGAGAATAAACAGATGGTAAAAGATGCGTTTATTCA  
1861 G N N Y H Y Y D E N K Q M V K D A F I Q

5641 AGATAGTGTGTTGGTAATTGGTATTACTTCGATAAAAATGGTAATATGGTTGCTAACCAAG  
1881 D S V G N W Y Y F D K N G N M V A N Q S

5701 TCCTGTTGAAATTAGTAGTAATGGAGCTTCAGGAACTTACCTTTTCTTGAACAATGGGAC  
1901 P V E I S S N G A S G T Y L F L N N G T

5761 ATCATTCGTTCTGGATTGGTGAAACTGATGCAGGTACGTACTATTATGATGGCGATGG  
1921 S F R S G L V K T D A G T Y Y Y D G D G

5821 CCGAATGGTTCGTAATCAAACGGTAAGTGATGGTGCGATGACATATGTTCTTGATGAAAA  
1941 R M V R N Q T V S D G A M T Y V L D E N

5881 TGGTAAACTTGTTAGTGAATCATTTGATTCTGCTACTGAAGCACACCCATTAAAACC  
1961 G K L V S E S F D S S A T E A H P L K P

5941 TGGTGATTTAAACGGCCAAAAATAATTACAATATGAAAATTGGAACCTTGATTTTACCTT  
1981 G D L N G Q K \* L Q Y E N W N L Y F T F  
inverted repeat

6001 CTTTGAAATAATATAGTTCTAATTAAGCAGCTCGCACCAAGACTTGGTATGAGCTGCTTT  
2001 F E I I \* F \* L S S S H Q D L V \* A A F

6061 TTTTGGCTCTACAATATCTGGTGTGATATAGAAATATCACTTTCTATACCAATATCAGA  
2021 F G S T I S G V D I E I S L S I P I S D

6121 TTTTGTTTTTTAAACTAAAAAAGAGGCTCGCCCTCTGATACAATGAAATCGCCAAATCAC  
2041 F C F \* T K K E A R P L I Q \* N R Q I T

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6181 ATAGTAAAGAAGGTAACCTCCATGGATAATGATACAAGAAGCTCTTCTCAATTTAACAGAC  
2061 \* \* R R \* P P W I M I Q E L F S I \* Q T

6241 CCTCATTTAAATTTTCCTCATCATTGGCTTAAATATAAAACAATTAAAAAGTTCGGGTG  
2081 L I \* I F L I I G L N I K Q L K K F G W

6301 GCACAAATATNCTGTACCCTTTCTTATACACCACGGGNCTTGTCCAAATTGGGGGAGTCA  
2101 H K Y X V P F L I H H G X C P N W G S H

6361 TTAATCGNGGTCAAATCTTAAATATGGGCTTTTATCAAGCTAAACACAATATGGACAAT  
2121 \* S X S N L K I W A F I K L N T I W T I

6421 TTAAAACTCAACCATTAATGNTG  
2141 \* N S T I N X

SEQ ID No. 13 DNA

SEQ ID No. 14 PRT

Lactobacillus reuteri strain ML1

1 ATCGATAATCAAATTGTTTATTTTGATATAAAGGAGATTAAATGGAAATAAAGAAACAT  
1 I D N Q I V Y F D I K E I K M E I K K H  
RBS start

61 TTTAAGTTGTATAAAAGTGGTAAACAATGGGTGACAGCGGCTGTTGCTACTGTGCGCGTT  
21 F K L Y K S G K Q W V T A A V A T V A V

121 TCAACCGCGCTTCTTTACGGGGGAGTTGCACATGCTGATCAACAAGTTCAGTCTTCCACA  
41 S T A L L Y G G V A H A D Q Q V Q S S T

181 ACTCAAGACCAAACCTTCTACTGTAAATACTAATACTACTAAAACAATAGCTGCAGATACT  
61 T Q D Q T S T V N T N T T K T I A A D T

241 AATGCTGATCAGCCAGCTCAAACAGCTGATAAAAATCAAGCAGCATCAAATGACACTACT  
81 N A D Q P A Q T A D K N Q A A S N D T T

301 AACCAAAGTAAAACTGATAGTACTTCAACAACCTGTTAAGAATCTTACTTCTACACCAGTT  
101 N Q S K T D S T S T T V K N L T S T P V

361 TCTACTTTGCCATCAACTGATAATGAAAAACAAAATCAAATTATAATAAGCATGATAAT  
121 S T L P S T D N E K Q N Q N Y N K H D N

421 GGAAACTATGGGAATATTGATACTGCTTACTTTAGCAATAATCAATTGCATGTTTCAGGA  
141 G N Y G N I D T A Y F S N N Q L H V S G

481 TGGAATGCAACGAATGCATCTCAAGGAACAAACAGTCGGCAAATTATTGTGCGTGATATC  
161 W N A T N A S Q G T N S R Q I I V R D I

541 ACAACCAATAATGAATTAGGTCGTACTGATGTAACAAACAATGTTGCGCGCCAGACGTT  
181 T T N N E L G R T D V T N N V A R P D V

601 AAGAATGTTTATAATGTTTATAACGCTGATAATTCTGGATTGATATTAATGTCAATATT  
201 K N V H N V Y N A D N S G F D I N V N I

661 GAATTTAGCAAGATGAAAGATTATCGGGATTCAATTGAAATTGTTAGTCGATACAGTGGA  
221 E F S K M K D Y R D S I E I V S R Y S G

721 AACGGTAAATCTATTGACTGGTGGTCCCAACCGATCACTTTTGACAAAAACAATTATGCT  
241 N G K S I D W W S Q P I T F D K N N Y A

781 TATCTTGATACATTTGAAGTGAAAAATGGCGAATTACATGCAACCGGATGGAATGCTACT  
261 Y L D T F E V K N G E L H A T G W N A T

841 AATAGTGCAATTAACATAATCACCATTTTGTAATTTTATTTGATCAAACGAATGGTAAG  
281 N S A I N Y N H H F V I L F D Q T N G K

901 GAAGTAGCACGACAAGAAGTTCGTGAAGGCCAATCACGCCAGATGTTGCTAAGGTATAT  
301 E V A R Q E V R E G Q S R P D V A K V Y

961 CCACAAGTAGTTGGTGCTGACAACCTCCGGCTTTGATGTGACATTTAATATCGGTAATTTA  
321 P Q V V G A D N S G F D V T F N I G N L

1021 GATTATACTCACCAGTACCAAGTTCTTAGTCGTTACAGCAATTCTGATAATGGCGAAGGC  
341 D Y T H Q Y Q V L S R Y S N S D N G E G

1081 GATAATGTTACCTACTGGTTTAATCCACAATCCATTGCTCCTGCTAATCAAAGTAACCAG  
361 D N V T Y W F N P Q S I A P A N Q S N Q

1141 GGTTATCTAGACTCATTTGATATTAGTAAAAATGGTGAAGTAACAGTGACCGGATGGAAT  
381 G Y L D S F D I S K N G E V T V T G W N

1201 GCTACTGACTTGTGAGAATTACAAAATAACCATTATGTAATTCTATTTGATCAGACAGCA  
401 A T D L S E L Q N N H Y V I L F D Q T A

1261 GGCAAACAAGTAGCATCTGCCAAGGCTGATTTAATTTACGTCCAGATGTTGCAAAGGCT  
421 G K Q V A S A K A D L I S R P D V A K A

1321 TATCCAACAGTAAAACTGCTGCAAATTCGGCTTTAAGGTAACATTTAAGGTTAATGAT  
441 Y P T V K T A A N S G F K V T F K V N D

1381 TTACAACCGGGTCACCAATATAGCGTTGTAAGTCGTTTCTCTGCCGATGAAAATGGTAAT  
461 L Q P G H Q Y S V V S R F S A D E N G N

1441 GGTAATGATAAGCGTCATACAGATTACTGGTTTAGTCCAGTAACATTAAACCAGAATGCT  
481 G N D K R H T D Y W F S P V T L N Q N A

1501 TCAAACATTGATACTATTACAATGACATCTAATGGGTTACATATTGGCAGTTGGATGGCA  
501 S N I D T I T M T S N G L H I G S W M A

1561 AGTGATAACTCAATTAATGAAACAACTCCATATGCTATTATTCTCAATAACGGTAAAGAA  
521 S D N S I N E T T P Y A I I L N N G K E

1621 GTTACTCGTCAAAGATGAGTTTAACTGCCCGTCCAGATGTAGCAGCAGTATATCCTTCA  
541 V T R Q K M S L T A R P D V A A V Y P S

1681 CTTTATAATAGTGCTGTTAGTGGGTTTGATACTACTATTAAATTGACTAATGATCAGTAT  
561 L Y N S A V S G F D T T I K L T N D Q Y

1741 CAAGCGCTTAATGGTCAATTACAAGTATTGTTACGTTTTTCTAAAGCTGCTGATGGTAAT  
581 Q A L N G Q L Q V L L R F S K A A D G N

1801 CCAAGTGGTGATAATACTGTAACGTATCAATTTAGTAAAAATTATGCAACTACTGGTGGA  
601 P S G D N T V T D Q F S K N Y A T T G G

1861 AACTTTGATTATGTAAAAGTAAATGGTAATCAAGTTGAATTTAGTGGTTGGCATGCAACT  
621 N F D Y V K V N G N Q V E F S G W H A T

1921 AACCAATCAAATGATAAAGATTACAATGGATTATTGTTTTAGTTAATGGTAAAGAAGTA  
641 N Q S N D K D S Q W I I V L V N G K E V

1981 AAGCGTCAATTAGTTAATGATACTAAAGAGGGGGCTGCTGGCTTCAACCGAAACGATGTC  
661 K R Q L V N D T K E G A A G F N R N D V

2041 TACAAAGTAAATCCAGCTATTGAAAACAGTTCTATGTCTGGATTCCAAGGCATTATTACT  
681 Y K V N P A I E N S S M S G F Q G I I T

2101 TTACCAGTAACAGTTAAGAATGAGAATGTTTCAGATTGTCCATCGTTTTAGTAATGATGCA  
701 L P V T V K N E N V Q I V H R F S N D A

2161 AAGACAGGTGAAGGTAGCCATGTTGATTTCTGGTCAGAAGTAATGCCAGTTAAGGATAGT  
721 K T \_ G E G S H V D F W S E V M P V K D S

2221 TTCCAAAAGGGTAATGGTCCGCTTAAGCAATTTGGCTTACAAACTATTAATGGTCATCAA  
741 F Q K G N G P L K Q F G L Q T I N G H Q

2281 TATTATATTGACCCAATGACTGGCCAACCTCGCAAGAACTTCCTATTACAAAATGGTAAT  
761 Y Y I D P M T G Q P R K N F L L Q N G N

2341 GACTGGCTTTTATTTTGATAATGAAACTGGTGAGGGAACTAATGCGTTAAAGAGGCAATTT  
781 D W L Y F D N E T G E G T N A L K R Q F

2401 GACGGAGGAACGATTTCTGCTGATAGTCAGTATAGAAAGGGTAATGAAGCTTATGGTTAT  
801 D G G T I S A D S Q Y R K G N E A Y G Y

2461 GACAATAAGAGCATTGAAAATGTTGATGGCTTTTAAACAGCTGATACTTGGTACCGACCA  
821 D N K S I E N V D G F L T A D T W Y R P

2521 AAACAAATTTTAAAATGGACCACCTGGACAGATTCTAAAGAAACAGATATGCGACCGCTC  
841 K Q I L K W T T W T D S K E T D M R P L

2581 TTAATGGTTTGGTGGCCAAATACTGTAACCCAAGCATATTACCTTAACTACATGAAACAA  
861 L M V W W P N T V T Q A Y Y L N Y M K Q

2641 CATGGAAACTTATTACCAGCTAATCTTCCATTCTTTAATTCTGATGCAGATCCATTAGAA  
881 H G N L L P A N L P F F N S D A D P L E

2701 TTAAATTATTATGCAGAAATTGTTTCAGCAAAATATTGAAAAGAAGATTAGTCAAACCTGGT  
901 L N Y Y A E I V Q Q N I E K K I S Q T G

2761 AATACTGACTGGTTGCGAACTTTGATGCACGAATTTGTATCTAATAATACAATGTGGAAT  
921 N T D W L R T L M H E F V S N N T M W N

2821 AAGAATAGTGAAAATGAAGACTTTGGTGGGTTGCAATTACAAGGTGGTTTTCTAAAGTAC  
941 K N S E N E D F G G L Q L Q G G F L K Y

2881 GTTAATAGTGATAAGACACCTAATGCTAATTCTAATTGGCGTATTATGGGTAGGCAGCCA  
961 V N S D K T P N A N S N W R I M G R Q P

2941 GCTAATATTGACGGAAATGGGCCAATTGGATCAGAATTCTTATTAGCTAATGACGTTGAT  
981 A N I D G N G P I G S E F L L A N D V D

3001 AATTCTAATCCAGTTGTTCAAGCTGAACAGTTAAATTGGCTACATTACTTATTGAATTTT  
1001 N S N P V V Q A E Q L N W L H Y L L N F

3061 GGAACTATTACTGCAAATGATCCTGATGCTAATTTTGATAGCATTTCGTGTTGATGCTGTT  
1021 G T I T A N D P D A N F D S I R V D A V

3121 GACAATGTAGATGCCGATTTATTAGATATAGCTGGTGATTACTTTAATGCAGTATATCAT  
1041 D N V D A D L L D I A G D Y F N A V Y H

3181 TCTCAAAGTAATGATAAAATTGCTAATGCTCATATTAATATTCTTGAGGATTGGGGTGGC  
1061 S Q S N D K I A N A H I N I L E D W G G

3241 CAAGATCCGTATTATACGCAAAGCATCGGAACCTCAATTATCGATGGATTATAATTTTC  
1081 Q D P Y Y T Q S I G T P Q L S M D Y N F

3301 TCAACTATAAGAAGTGTGTTAGCATCTAACACTGCATCAATGACTGATATTATTAAGAAT  
1101 S T I R S V L A S N T A S M T D I I K N

3361 TCATTGGTAAATCGGAGCTTAGATAATGCTGAAAACGTATCAATTCCTAATTACTCATTT  
1121 S L V N R S L D N A E N V S I P N Y S F

3421 ATCCGTGCACATGATAATGGTTTCAAGATGATATTAAGCGTGCAATTTTCAGATGTAAAT  
1141 I R A H D N G S Q D D I K R A I S D V N

3481 AATTTACCATATGGTTTGAAGTTTAACTTTGAGCAAGAGCAAAAGGGGATTGAAGCATAC  
1161 N L P Y G S K F N F E Q E Q K G I E A Y

3541 ATTGCAGATCAAAGTAATGTTAATAAGAAGTGAATAATTATAATATTCCATCTTCATAT  
1181 I A D Q S N V N K K W N N Y N I P S S Y

3601 GCTATTATGTTGACTAATAAGGATACCGTTCCTCGTGTATATTATGGTGATTATTTACT  
1201 A I M L T N K D T V P R V Y Y G D L F T

3661 GATGGTGGTCAGTATATGGCACAAACAACGCGTTATTATCCTGCACTTACAAGTCTTTTA  
1221 D G G Q Y M A Q T T R Y Y P A L T S L L

3721 AAGGCACGTATTAAGTATGTAGCTGGTGGACAAACAATGTCTGTGCGATAAGAATAATATT  
1241 K A R I K Y V A G G Q T M S V D K N N I

3781 TTGACTAGTGTTCGCTTTGGTAAAGGTGCGATGAATCCTACTGATATGGGTGATAGTTTA  
1261 L T S V R F G K G A M N P T D M G D S L

3841 ACTAGAACATCTGGTGTGGGGTAGTTATAAGTAATAATGATAAATTATTATTAAGCTCA  
1281 T R T S G V G V V I S N N D K L L L S S

3901 AATGATAAAGTTGTATTACACATGGGTGCTGCACATAAGAATCAGAAATTTAAAGCAGTC  
1301 N D K V V L H M G A A H K N Q K F K A V

3961 TTACTAACTACTAATGATGGTATTTCAGAGTTTTAATGATGACAATGCGCCTGTTGCATAT  
1321 L L T T N D G I Q S F N D D N A P V A Y

4021 ACTGATGCTAATGGTGACTTGGTCTCTTCTGGTAAAGATATTACGACTGATGGTGTAATT  
1341 T D A N G D L V L S G K D I T T D G V I

4081 CAACATAATACTGCTGTTAAGGGCTATGCTAATGCTGATGTAAAGGTTATCTTGCAGTA  
1361 Q H N T A V K G Y A N A D V K G Y L A V

4141 TGGGTTCCAGTAGGTGCCAGTGATACAACAGGATATTAGAACAGCACCATCAGGGGTACAA  
1381 W V P V G A S V Q Q D I R T A P S G V Q

4201 AGTGATGGAAAGTCTGTTTATCATTCAAATGCAGCTCTGGATTCAAATATTATTTTGA  
1401 S D G K S V Y H S N A A L D S N I I F E

4261 GGATTCTCTAACTTTGTATATTGGCCGACAAATAATTCTGAGCGTGCAAATGTAAAAATC  
1421 G F S N F V Y W P T N N S E R A N V K I

4321 GCTCAGAATACTGACTTATTTAAGGAGTTGGGTATTACTTCATTTGAATTAGCTCCACAG  
1441 A Q N T D L F K E L G I T S F E L A P Q

4381 TATAATTCAAGTAAGGATGGCACATTCCTTGATTCTCAGATTGATAATGGATATGCATTT  
1461 Y N S S K D G T F L D S Q I D N G Y A F

4441 ACTGATCGCTATGATCTAGGTATGAGCATTCCAAATAAGTATGGTAGCGATACTGATCTA  
1481 T D R Y D L G M S I P N K Y G S D T D L

4501 AGGAATGCTATTAAAGCCTTACATAAGGCCGAATTCAAGCAATGGCTGATTGGGTTTCCT  
1501 R N A I K A L H K A G I Q A M A D W V P

4561 GATCAAATTTATAATTTACCAGGTAAAGAAGTTGTTACTGCTACTCGTGTGGACGAACGT  
1521 D Q I Y N L P G K E V V T A T R V D E R

4621 GGAAATGATTGGAATGTAGCTCAGATTAAGGATTCATTTATGTTGCTAATACAATTGGT  
1541 G N D W N V A Q I K D S L Y V A N T I G

4681 GGTGGAAAGTATCAAGAGCAATATGGTGGAGCTTTCCTTGATCAATTACAAAAGCAATAT  
1561 G G K Y Q E Q Y G G A F L D Q L Q K Q Y

4741 CCACAAATCTTTGAACGTAAACAACCTTCAACTGGTGTAGCAATTGACCCAAGTACTAAG  
1581 P Q I F E R K Q P S T G V A I D P S T K

4801 ATTAAACAGTGGTCTGCTAAATACTTTAATGGGACAAATATTTTACATCGTGGTGCAGGG  
1601 I K Q W S A K Y F N G T N I L H R G A G

4861 TATGTATTAAGAGATAACGGTGGTAACTACTTTAGCCTTGGAATAGTAATAATAACAG  
1621 Y V L R D N G G N Y F S L G N S N N K Q

4921 TTATTACCAAATCAATTATCAGGTAAGGCTGAAAATGGCTTTGTTGATGTTAATGGGAAT  
1641 L L P N Q L S G K A E N G F V D V N G N

4981 ACTAAATACTTTACATCAACCGGAATTCCTGTACGGATGCATTTGTTCAAGACAGTGTA  
1661 T K Y F T S T G I P V T D A F V Q D S V

5041 GGTAAGTGGTACTATATTGATAAAAATGGTAATATGCTTAAAAATACCGGTTTTGTAGAT  
1681 G N W Y Y I D K N G N M L K N T G F V D

5101 ATTACGCGAAATGGTCAGACAGGTACGTATCTATTCTTAAATAACGGTATCTCATTCCGA  
1701 I T R N G Q T G T Y L F L N N G I S F R

5161 TCAGGATTAGTTAAAATTGGTAATGATACTTATTACTTTGACGGTAATGGAAAAATGGTT  
1721 S G L V K I G N D T Y Y F D G N G K M V

5221 CGTGGCCAATCTATTAGTGATGGTACGATGAATTATACTCTTGATAAGGATGGTAAATTA  
1741 R G Q S I S D G T M N Y T L D K D G K L

5281 GTTGGCTTGTTATTATGATCCAAGTAGTCAGAATCCACATCCAATTACTCAACAGGATTTA  
1761 V G L Y Y D P S S Q N P H P I T Q Q D L

5341 AGTGGTACTAATAAGTAGTTTATTAAAAATCACCAATAGAAGTTGTCTCTACATCAAATG  
1781 S G T N K \* F I K N H Q \* K L S L H Q M

5401 GTGTTGATATGAAAATATAATACTTTTATACCATTAAATTGGTCTAGTAAGAATCATCCTC  
1801 V L I \* K Y N T L Y H \* I G L V R I I L

5461 ACGGATGGTTCTTTTTAGTTTCGCCGTTTGTAATAAAGTTAGAAAAAATAAAAAGCCA  
1821 T D G S F \* F R R L \* N \* V R K N K K P

5521 TTTGTGATAGACTTTTGAGTATCCCTAATCAAAAGAAAGGCAATCACAAATGACCTATAA  
1841 F V I D F \* V S L I K R K A I T N D L \*

5581 ACATCTTACCACACGCGAATTAACCTCTCATAGCTGATTTTTGGTATCAAGGCACTAAAGC  
1861 T S Y H T R I N S H S \* F L V S R H \* S

5641 TTATCGGGCTGCTAAATTACTTCAACGTAGTCAAGAAACCATCTATCGTGTATTATCGTTT  
1881 L S G C \* I T S T \* S R N H L S C L S F

5701 CCTCAATAACGGTAAAACCATCGACCAATATCTTCAGACTTATCAGCGACATAAACGTCG  
1901 P Q \* R \* N H R P I S S D L S A T \* T S

5761 TTGTGGTCGGAAGCAGACCCAACTGCCAACTATCGAGGTTAACTATATCCATGCGCAAAT  
1921 L W S E A D P T A N Y R G \* L Y P C A N

5821 CAAGGCTGGTTGGACTCCTGATACTATTATTGGTCGTGATGAGCACCCGATTAGCTGCAG  
1941 Q G W L D S \* Y Y Y W S \* \* A P D \* L Q

5881 ATACTAATGCTGATCAGCCAGCTCAAACAGCTGATAAAAATCAAGCAGCATCAAATGACA  
1961 I L M L I S Q L K Q L I K I K Q H Q M T

5941 CTACTAACCAAAGTAAAACTGATAGTACTTCAACAACTGGTAAGAATCTTACTTCTACAC  
1981 L L T K V K L I V L Q Q L V R I L L L H  
6001 CAGTTTTCTACTTTGGCATCAACTGATAATGGAAAACAAAATCAAATTATAATAAGCAT  
2001 Q F S T L A S T D N G K Q N Q N Y N K H  
6061 GATAT  
2021 D

SEQ ID No. 15 DNA

SEQ ID No. 16 PRT

Lactobacillus reuteri strain ML1 (ML4)

1 AATATTGATGGTTACTTAAGTTATACTGGTTGGTATCGTCCTTATGGAACGAGTCAAGAT  
1 N I D G Y L S Y T G W Y R P Y G T S Q D  
61 GGTAAAACATGGTACGAAACAACCTGCAATGGATTGGCGTCCATTACTGATGTATATTTGG  
21 G K T W Y E T T A M D W R P L L M Y I W  
121 CCAAGCAAAGATGTTCAAGCACAATTTATTAAGTATTTTGTTAATAATGGTTATGAGAAT  
41 P S K D V Q A Q F I K Y F V N N G Y E N  
181 GCTAATTATGGACTTACAGAGTCCTCTGTTGCTTCCTTTAGCAAGGATACTAATGCTAAT  
61 A N Y G L T E S S V A S F S K D T N A N  
241 CTCCTCGATGTAACCTGCACAAAATCTTCGTTATGTAATTGAGCAAAGTATTGCAGCCAAT  
81 L L D V T A Q N L R Y V I E Q S I A A N  
301 AAAGGGACAAGTAAGTTAGCAAATGATATTAATAGTTTTGCTGCAACGGTTCCTGAATTA  
101 K G T S K L A N D I N S F A A T V P E L  
361 TCTGCATCATCTGAATTATCATTGCAAAGCATGCCAACTATCGACCAGATGAAAGTGGGA  
121 S A S S E L S L Q S M P N Y R P D E S G  
421 ACTGTTGATAGTGATCAAGTCATTTTGTTAATAATAATTCAAAGGATCCCCGTAAAGGG  
141 T V D S D Q V I F V N N N S K D P R K G  
481 AACACTGGTTATGCGGACAGCAACTATCGCTTAATGAACAGGACGATTAATAATCAGGCC  
161 N T G Y A D S N Y R L M N R T I N N Q A  
541 GGAAATAATAATAGTGATAACAGTCCAGAACTCCTTGTTGGTAATGATATTGATAATTCA  
181 G N N N S D N S P E L L V G N D I D N S  
601 AACCCAGTAGTACAAGCTGAAAATCTTAATTGGGAATACTTTTTACTAAATTATGGTAAG  
201 N P V V Q A E N L N W E Y F L L N Y G K  
661 TTAATGGGGTATAATCCAGACGGTAATTTTGATGGCTTCCGAGTTGATGCTGCTGATAAT  
221 L M G Y N P D G N F D G F R V D A A D N  
721 ATTGATGCAGATGTCTTAGATCAAATGGGTCAATTAATGAACGACATGTATCATACAAAG  
241 I D A D V L D Q M G Q L M N D M Y H T K  
781 GGAAATCCTCAAAATGCAAATGATCATTGAGTTATAATGAAGGTTATCATTCTGGGGCT  
261 G N P Q N A N D H L S Y N E G Y H S G A  
841 GCACAAATGCTAAATGAAAAGGGTAATCCTCAATTGTACATGGATTTCAGGCGAATTCTAT  
281 A Q M L N E K G N P Q L Y M D S G E F Y  
901 ACCCTTGAGAATGTTCTCGGACGTGCAAATAACCGTGATAGTATCGGTAATTTAATTACT  
301 T L E N V L G R A N N R D S I G N L I T



961 AATAGTGTGTTAATCGGCAAAATGATACAACAGAGAATGAAGCTACGCCAAACTGGTCA  
321 N S V V N R Q N D T T E N E A T P N W S

1021 TTTGTAACCTAACCATGATCAACGAAAGAATTTGATTAATAGATTAATTATTAAGGGTCAT  
341 F V T N H D Q R K N L I N R L I I K G H

1081 CCTAACATTCCGGATATTATGGGTTTACAAAGCTGAATATGCAATCAAGCATGG  
361 P N I P D I M G S A Y K A E Y A N Q A W

1141 CAAGAATTCTACGCTGATCAGAAAAAGACTAATAAACAATATGATCAATATAATGTTCCG  
381 Q E F Y A D Q K K T N K Q Y D Q Y N V P

1201 GCTCAGTATGCAATTCTTTTGAGCAATAAAGATACGGTTCCGCAGGTTTACTATGGTGAC  
401 A Q Y A I L L S N K D T V P Q V Y Y G D

1261 CTTTATAATGAACTGCTCAATACATGCAAGAGAAGTCAATTTACTATGATACAATCAGC  
421 L Y N E T A Q Y M Q E K S I Y Y D T I T

1321 ACTCTTATGAAGGCCCGTAAACAATTTGTTAGTGGTGGTCAAACGATGACTAAACTTAAC  
441 T L M K A R K Q F V S G G Q T M T K L N

1381 AATAATTTATTAGCTAGTGTTCGATATGGTAAGGGTGTGCTGATTCTAATAGCAATGGT  
461 N N L L A S V R Y G K G V A D S N S N G

1441 ACCGATAAGCTTAGCCGAACAAGTGGGATAGCCGTCTTAGTTGGTAATGATAGTAATATG  
481 T D K L S R T S G I A V L V G N D S N M

1501 GCTCAACAACTGTTGCTATTAATATGGGTCGCGCTCATGCTAACCAACAATATCGAAAT  
501 A Q Q T V A I N M G R A H A N Q Q Y R N

1561 TTAATTGATACTACCGAAAATGGCTTGACATATGATGGAGAAAATAGTGAAAATCCAGCC  
521 L I D T T E N G L T Y D G E N S E N P A

1621 ATTTTGACAACTGATAGTAATGGTATCTTAAAAGTAACAGTTAAAGGATACAGTAACCCA  
541 I L T T D S N G I L K V T V K G Y S N P

1681 TACGTAAGTGGTTATCTTGGTGTGTTGGGTTCCAGTAATTTCTGGTGATCAAGATGTTACT  
561 Y V S G Y L G V W V P V I S G D Q D V T

1741 ACAAGTGCAAGTGATGTTGTTGCTGATAAAGAAAAGACTTTTGAATCTAATGCTGCTCTT  
581 T S A S D V V A D K E K T F E S N A A L

1801 GATTCTCATATGATCTATGAAGATTTTACGCTTGTTCCAACCAGAACCAACTAATGTTGAG  
601 D S H M I Y E D F S L F Q P E P T N V E

1861 AATCATGCTTACAATGTGATTGCTAAAAATGCTAATCTCTTTAATGATTTAGGCATTACT  
621 N H A Y N V I A K N A N L F N D L G I T

1921 GATTTTTGGATGGCTCCTGCTTACACTCCATTTGGAATGAGTCGTTATAATGAAGGATAC  
641 D F W M A P A Y T P F G M S R Y N E G Y

1981 TCAATGACGGATCGTTACAATTTAGGTACGACAGCTAATCCAACAAAGTATGGTAGTGGA  
661 S M T D R Y N L G T T A N P T K Y G S G

2041 GAAGAGCTTGCAAATACAATTGCTGCATTGCATAAAGTAGGATTAAAAGTTCAAGAAGAT  
681 E E L A N T I A A L H K V G L K V Q E D

2101 ATTGTTATGAATCAGATGATTGGTTTCTCTGGTCAAGAAGCAGTAACGGTTACTCGAACA  
701 I V M N Q M I G F S G Q E A V T V T R T

2161 AATAATCGTGGAATGCAGATTCATGTAAATGGTCAAACATATGCAATCAAATTTACTTT  
721 N N R G M Q I H V N G Q T Y A N Q I Y F

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2221 GCATATACAACCTGGTGGCGGAAATGGTCAAGAACTTATGGTGGTAAATACCTTGCCGAA  
741 A Y T T G G G N G Q E T Y G G K Y L A E

2281 TTACAAAAGAACTATCCTGACCTATTTACGACCAAGGCAATTTTCGACAGAAGTTGTACCT  
761 L Q K N Y P D L F T T K A I S T E V V P

2341 GATCCAACCGTTTCGTATTAAT  
781 D P T V R I N

SEQ ID No. 17 DNA

Lactobacillus strain LB33

1 ATGGAATTAA AAAGGCATTA CAAGATGTAC AAGGCTGGTA AAAAATGGGT TTTTGCTGCA  
61 ATTGCCACAA TCTCTATAAT TGCAGGATTA AATACAGTGG CAGTGACAAC CTATGCTGCC  
121 GGCAATAATG ATCCGCAGCA GACCACTACT CAAAATGCAC CTAACCAACAG TAACGATCCG  
181 CAATCTACTA CTACGCAGAA TACTGCCAAC AACAGTAACG ATCCGCAATC TACTACTACG  
241 CAGAATACTG CCAACAACAG TAATGGTCCA CAATCTACTA CTACGCAGAA TACTGCCAAC  
301 AATAGTAATG GTCCACAATC TACTACTACG CAGAATACTG CCAATAACAG TAACGATCCA  
361 CAATCTACTA CTACGCAGAA TACTGCCAAC AACAGTAACG ATCCGCAATC TACTACTACG  
421 CAGAATACTG CCAACAATAG TAATGGTCCA CAATCTACTA CTACGCAGAA TACTGCCAAC  
481 AACAGTAACG ATCCGCAATC TACTACTACG CAAAACACTG CCAACAACGG TAATGATCCA  
541 CAATCTACTA CTGGAAAAGA TACAGTTAGT ATTGCAGATA TTCAAGTTAA CCAACCTGTT  
601 AATCTTTTAG GAAAGCAATC AACTGTATCT AGTACTGGTT ATAATGACTC TCACATAAAA  
661 AATGTCAATG GGAAAATCTA TTTTGTGGT GATAATGGTC AGGTCAAGAA AAACCTTACA  
721 GCCATAATCA ATGGACAATC ACTATATTTT AATAAAACAA CTGGAGAATT GGCTTCTAAT  
781 GATGTTCAAT ATGAAAATGG GTTAGTAAAA ATAAACGATG TTCATAACGC CGCTTACTCT  
841 ATTGATCCAA CGGGATTAC TAATGTTAAC GGATTTTTAA CTGCTAATAG TTGGTATAGA  
901 CCCAAATATA TTTACAAAGA TGGGCAAAAA TGGGTGGAAT CAACCTCTCA AGATATGCGT  
961 CCCCTTTTAA TGACATGGTG GCCAGATAAA AATACTCAAG TAGCTTATTT ACAATATATG  
1021 CAGAAAATGG GCATTTTACC CGCTGACGTC ACTATATCAA GTCAAACCAA TCAATCAGTT  
1081 TTAACCAAAG AATCATTAT TACTCAAGCT GAAATTGAAA AACAGATTGG AGTAACAAAT  
1141 GGAAACACTG ATTGGCTAAA GAAAGATATC TCTGATTTTG TAAATTCTCA ACCAAATTGG  
1201 AATATAGATA GTGAAGCCAA AGGCACAGAC CATTTCAGG GGGGAGCACT TTTATATGTT  
1261 AATAATAAGT TAATCCATA TCGAATTCT GATTACCGCT TGCTTAACCG AACACTTACT  
1321 AATCAACAGG GGCAAGTAAA AGATACTTCT AAACAAGGCG GTTATGAAAT GTTACTTGCC  
1381 AACGATGTGG ATAATTCCAA TCCAGTAGTT CAAGCGGAAC AGTTAACTG GTTATACTAC  
1441 ATGATGAATA TAGGTAGCAT TACTGCCAAT GATCCACCG CAACTTTGA TGGCTATCGA  
1501 GTGGACGCTG TGGACAATGT CGATGCTGAT TTATTAAATA TAGCTGCCGA TTATGCCAAA  
1561 GATGCTTATA AAATAATCA AAGTGATGCT AATGCCAACA AACATTTATC AATATTAGAA  
1621 GATTGGGATA ATAATGATCC GGCTTATATC AAAGCACATG GAAATAATCA GTTAACATG  
1681 GATTTCACAG CACATTTAGC AATTAAATAT TCATTAAATA TGCCAGTAAG TCAACGAAGT  
1741 GGGCTGGAAC CAGAGCTCAC AACCAGTTTA GTTAACAGAA CTGGTGATGA TTCTACTGAA  
1801 AATGTCGCAC AGCCAAACTA TACTTTTATT AGGGCTCACG ATAGTGAAGT GCAAACAATC  
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1921 GATGAAATAA GTCAGGCCTT TAAATATAT AATGCAGATG AATTAAAGAC TGATAAACAA  
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2101 CCTTACTATG ATGCAATAAC TACGTTATTA AAAACACGAA TGAAATACGT ATCTGGTGGT  
2161 CAAAACATGC GTATGCAATA TATGCAGGGT GATGATATGC CTGCTAATAG CTATAAGGGC  
2221 GTTTTAACTT CAGTTAGATA TGGTAAGGGT GAAATGACAG CCGATGAGCA AGGTAATTCA  
2281 GAAACTCGTA CTCAAGGAAT TGGGGTCATT ATAAGCAATA ATCCTAATTT AAAATTAGAC  
2341 AGTAATGACC AAGTGGTATT AAATATGGGG GCGGCACATG AAAATCAAAC TTATCGCCCT  
2401 GTATTACTAA CAACTAAAGA TGGATTGAAA AACTATGATT CCGATAGTTC TGTACCTCAA  
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2581 GATAATCAAG ATGCTCGGAC TGCAAGCAGT TCTCAGCCAT CAACTGATGG GAAAACATAT  
2641 CATTCCAATG CTGCTTTAGA CTCTCAAGTT ATTTACGAAG GATTTTCTAA TTTTCAATCG  
2701 ATTCTTACAA ATACAGAAGA TTTCACTAAT GTAAAAATTG CTCAAAACGC TAACCTGTTT  
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2881 TATAATACTC CGACAAAATA TGGAACTGTT ACTCAATTGC TGGATGCATT AAGGGCTTTA  
2941 CATGCCAACG GAATTCAGC GATCGATGAC TGGGTTCCCTG ACCAAATATA CAATTTACCT  
3001 GGTGAGGAAA TTGTCGCAGC TCAAAGAACT AATGGATCTG GGACATATGA TCAAGATTCT

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3061 GTTATTGATG ATACATTATA TGATTCTCAC ACTGTTGGTG GTGGCGAATA TCAAGCTAAA
3121 TTTGGTGGAG CTTTTCTAAA CAAGTTAAAG CAGTTGTATC CTGATTTATT TAAAGTTAAA
3181 CAAATTTCTA CTGGTCAACC TATGAATCCT AATGAAAGAA TTACCGAGTG GTCAGCAAAG
3241 TACTTTAATG GTACAAATAT TCAAGGAAGA GGCGCTTGGT ATGTATTAAA AGACTGGGGT
3301 ACCAATCAGT ACTTTAATGT AAGTAATAAC CAGTTGTTC CCAAACAATT CCTAGGTACA
3361 GATACTTATA CAGGCTTTAA TGTTACAAAT GAGGGAACTC AGTTTTATT CACGAGTGGG
3421 TATAAAGCCC AGAATACCTT TATTCAGGAC GGAGACAACT GGTATTACTT TGACAATAAT
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3781 AAAGATAATA ATGGTAATTT AAGATATTTT GACGGTAATA CAGGTGATAT GGTCAATTAAT
3841 TCATTTGGAG AACTTCCTGA TGGCTCTTGG TTATACCTTA ATGATAAGGG GATTGCCGTT
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3961 AAGAATGATT TTAGAGAGTT GCCTGATGGT TCATGGCTTT ATCTTAATGA CAAGGGGATT
4021 GCCGTTACTG GTAAACAGGA AATCAATGGT CAAACCTACT ACTTTGATGC GGATGGCAAG
4081 CAAGTGAAGA ATGATTTTAG AGAGTTGCCT GATGGTTCAT GGCTTTATCT TAATGACAAG
4141 GGGATTGCCG TTACTGGTAA ACAGGGAATC AATGGTCAA CCTATGCAGA GGCTAAAATC
4201 ACAGCTGCCG AAAATGCTCA TCAAGCTGCC ACAGACGCTG TGAATAAAGC CCAAGCTGCT
4261 CAATCGCCTA ACACTAGTTC CTCTAGTTCT AGCGTTAGCC AAGCTACTAA ACATCAATTG
4321 GCAGTTAAAA CTGCTAAAGC TCAACTTGCT AAAACTAAGG CTCAAATTGC TAAGTATCAA
4381 AAGGCTTTGA AAAAAGCCAA AACTACAAAG GCCAAGGCTC AAGCTCGTAA AAGTTTGAAG
4441 AAGGCCGAGA CTAGTTTCAG CAAAGCTGAA CTTAATTTGG CATTATTAAA TAATAAAGCC
4501 GTAAAAGCTG CACAAACTAA GGTAAATAAG GCTAAGGCTC AAGTCACTAA ATACCAAAG
4561 GCTTTGAAGA AAGCTAAGAC TACAAAGGCT AAGACTCAAG CTCGTAAAAA TTTGAAGAAG
4621 GCCAACTCTA GTCTGACAAA AGCTCAAAAA GCATTAATAA AAGTAATTAA AACCAATATC
4681 AAGTAA

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SEQ ID No. 18 PRT

Lactobacillus strain LB33

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MELKRHYKMYKAGKKWVFAA IATISIIAGLNTVAVTTYAA
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QNTANNSNGPQSTTTQNTAN NSNGPQSTTTQNTANNSNDP
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SEQ ID No. 19 DNA

SEQ ID No. 20 PRT

Lactobacillus sake strain KG15

1 SASCTGBCMSTNACGTTHRRCNTAGACGTTHRACGTACTGGTTCACACAATGGATTCCGGC  
1 X X X X R X X X T X X V L V H T M D S A  
61 AAACATCAATGATTGCGATCTGTCCAGGTTGGGCTGCTTCACGCGTCAAACCGTACGG  
21 N Y Q \* L R S V Q V G L L H A S N Q Y G  
121 ATCGCATTGACCACGGGTAATAATTGTAGTGC GCGACGGTTGAACCGTGACCGACTAATG  
41 S H \* P R V I I V V R D G \* T V T D \* W  
181 GTGATTTTTTTCGGGCATAAAGGCGGTCAATCAAGCGCCAAAACGGCGTTGTGATTGAATA  
61 \* F F A A \* R R S S S A K N G V V I E Y  
241 CCAAGCGTTGTTTGTAACACAGTAGCGCCAACAATCGACAGTCATCGATTTTAACGTGC  
81 Q A L F V N T V A P T I D S H R F \* R A  
301 GCCACATTACGCCGTTGCGTCACACAACGTGGGCAATAGCGCTGGTAAAGCGACTGGCAC  
101 P H Y A V A S H N V G N S A G K A T G T  
361 AGCTGATAAAAATAATGATAGTTACCTTGTAATTCGTGACGAATTTGTTTAACTTAGGA  
121 A D K N N D S Y L V I R D E F V \* T \* D  
- 35  
421 TGGTTCAACATCGTTAGGACCCCTTTTAAGTTTAGTCACTTATGAATCTAACTGTGTGG  
141 G S T S L G P L L S L V T Y E S N C V G  
- 10  
481 ACTTTTTTGTTAATTTTTTTGTATTATTACAACTAGCACCACGCGTATGTGTTTTATTA  
161 L F C \* F F C I I T N \* H H A Y V F Y \*  
RBS  
541 ATACCACTTAATTAATAACGGGGCTTTAGCATGATTTCAAATAAAATAGTGTGAAAGGTA  
181 Y H L I N N G A L A \* F Q I K \* C E R \*  
start  
601 GTTTTTTATGTTAAGGAATAATTATTTTGGAGAGACTAAAACGCATTATAAATTATATAA  
201 F F M L R N N Y F G E T K T H Y K L Y K  
661 ATGCGGTAAGAACTGGGCTGTCATGGGGATTTTCAATTTCCGCTGGGATTAGGGATGCT  
221 C G K N W A V M G I S L F P L G L G M L  
721 AGTTACCAGCCAGCCAGTGTGCTGATGTGACAGCCACCAGCACCTCAAGCAGTGCAGT  
241 V T S Q P V S A D V T A T S T S S S A V  
781 GAGGACCGATGCAATCAGTGCAAGTAGTAGCAGTGCAGCAAAGGCTGAAACGGCTGCGAT  
261 R T D A I S A S S S S A A K A E T A A I  
841 CACTACTGCAGGTGTTGCAAATGCTGATTCACAAACATCAGCAGAAGTAACCGCTGACTC  
281 T T A G V A N A D S Q T S A E V T A D S  
901 TACTTCTACCAGCCAAGTGGTAATAAATTCCAATAATCAAAATAATACAGCACAGCC  
301 T S T S Q V V T N N S N N Q N N T A Q P  
961 AGCCGGTCAAGAAGCAGCCCCGGTATCAGAGGACACATCATCTGATGATAGTGAGAGAAC  
321 A G Q E A A P V S E D T S S D D S E R T

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2281 TCAGGGTGTATGGTAACGGGTAAGCAACGTGTGCACCAAGATCAGTATTTCTTCCTGCC  
761 Q G V M V T G K Q R V H Q D Q Y F F L P

2341 AAATGGTATTGCTTTGACAGATGCTTTTCGTACAAACTGCTGATGGTCAACGTCAGTACTA  
781 N G I A L T D A F V Q T A D G Q R Q Y Y

2401 TGATAAAACAGGTCGTCTGGTCATTAATCAATATGTGACTGACCACCAAGCGAATGCGTT  
801 D K T G R L V I N Q Y V T D H Q A N A F

2461 CCGGGTTGATGCAGACGGTAACGTTGTCCGCAATCAAGCTTTGACTGTTGACGGCCATGA  
821 R V D A D G N V V R N Q A L T V D G H E

2521 ACAATATTTCCGGCACAACGGTGTCCAAGCGAAAGCAGTGCTCATTGCAACTGACGATAA  
841 Q Y F G T N G V Q A K A V L I R T D D N

2581 TCAGGCGCGCTACTACGAAGCCAATAGTGGTAATCTCGTGAAGCAACAGTTTATTCTTGA  
861 Q A R Y Y E A N S G N L V K Q Q F I L D

2641 TACAGATGGACATTGGTTGTACGCGGATGCTGCAGGTGACTTGGCACGCGGACAAATTAC  
881 T D G H W L Y A D A A G D L A R G Q I T

2701 AATTGGCCAAGACACGTTGTATTTTGATGATAATAATCACCAGGTAAAAGATGATTTTCGT  
901 I G Q D T L Y F D D N N H Q V K D D F V

2761 CTATGATACTAACGGTGTGCATTATTTTAATGGCACAACAGGCGCTGAAATCAAACAAGA  
921 Y D T N G V H Y F N G T T G A E I K Q D

2821 TTACGCGTTTCATGATGGCAAATGGTACTATTTTGATGATTTGGGACGAATGGTAACCGG  
941 Y A F H D G K W Y Y F D D L G R M V T G

2881 CTTGCAGCGTATTAATGGTGAGTATCGCTATTTTGATGCTAATGGTGTGCAACTAAAGGG  
961 L Q R I N G E Y R Y F D A N G V Q L K G

2941 CGGTACCGTGACCGATCCACTAACGCACCAAACGTACACTTTTGATGCGAAAACCTGGTGC  
981 G T V T D P L T H Q T Y T F D A K T G A

3001 TGGTACGTTGGTGACGATTTAACTGAATAATGGACTAGAAAAGACGATCTTGTATCGTCT  
1001 G T L V T I \* L N N G L E K T I L Y R L

3061 TTTT\*AGTTTCGATAACTAAATAAGTGCTCATTTT\*GCATTAGGACTCAGAATTAGCGGG  
1021 F \* F R \* L N K C S F L H \* D S E L A G

3121 CGCGCAAGCGTCTTTTCGTGTTAACTTATTAGTAATTAATATTTTGAGGAGTCTGTTAT  
1041 A Q A S F R V K L I S N \* Y F E E S V I

3181 ATGGCAACAATTTTAGTTGTAGATGATGAACCGTCATTGGTGACGCTACTGTCATACAAC  
1061 W Q Q F \* L \* M M N R H W \* R Y C H T T

3241 CTGACTAAATCAGGCTTCGAGGTCGTGACTGCTACCTCCGGTGACGAGGCACGAAATCAG  
1081 \* L N Q A S R S \* L L P P V T R H E I S

3301 CTGGCAAATCATCCTATTGATTTGATGCTGCTAGGTGTCATGTTGCCTGGTAAGAGTGGC  
1101 W Q I I L L I \* C C \* V S C C L V R V A

3361 GTTGACTTAACACGAGAACTACGAGGCGAACAAGAATCGTATTCCAATTATTATGATTACC  
1121 L T \* H E N Y E A N R I V F Q L L \* L P

3421 GCCTTGGATGACGAAGTTGACAAGATTT  
1141 P W M T K L T R F